

Elements in foraminiferal shells as recorders of past climates

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Elements in foraminiferal shells as recorders of past climates

Elementen in de schelpjes van foraminiferen als afspiegeling
van het klimaat in het verleden
(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de
Universiteit Utrecht
op gezag van de rector magnificus,

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ingevolge het besluit van het college voor promoties
in het openbaar te verdedigen op

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Promotor:
Prof. dr. G.-J. Reichart

Co-promotor:
Dr. L.J. de Nooijer

"The global Pleuston is exquisitely adapted for the perpetually flat world in which it exists. Upward and downward trips into other realms of existence are rare and imperfect. Pleuston dwellers are largely unresponsive in body and instinct to the rest of reality. The skin of water, along with what comes in and what goes out, is the universe they know".

- The origin of creativity, Edward O. Wilson

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SEM images courtesy of Inge van Dijk

Chapter 1

Introduction and synopsis

"Please don't break this shell that I call home"

- Ophelia, Marika Hackman

1.1 Reconstructing climate change using foraminiferal shells

Earth's climate varied greatly throughout geological times, with large shifts over the course of hundreds of millions of years. This resulted in alterations between 'ice-house' and 'green-house' climate states, whereby tectonic processes played a large role. On shorter timescales (e.g. during the most recent ice-house climate of last 3 million years), orbital forcing paced glacial and interglacial episodes. Within these periods, the climate showed even more rapid fluctuations due to non-linear feedbacks and teleconnections between different areas around the globe. For example, the Younger-Dryas, a relatively short return to cooler conditions during the last deglaciation, between 12,900 and 11,700 years ago, was marked by an abrupt warming transitions within only 1-3 years (Steffensen et al., 2008) and is a well-documented example of the interplay between the strength of the Atlantic overturning circulation and the monsoon system (Broecker et al., 2010; Steffensen et al., 2008). Hence, climate change is an inherent feature of the climate system. Understanding the processes involved in (rapid) climatic fluctuations in the past provides a mechanistic framework to better predict future global change.

Since the onset of the Pleistocene, 2.58 million years ago, Earth has been in an ice-house state, with orbital motions pacing the waxing and waning of ice sheets in the northern hemisphere. A compilation of sediment records showed recurring variations in Earth's climate with cycles of 23,000, 42,000 and approximately 100,000 years (Hays et al., 1976), reflecting Milankovitch cycles in Earth's precession, obliquity and eccentricity respectively (Imbrie et al., 1984). These climate fluctuations are accompanied by changes in atmospheric $p\text{CO}_2$ levels (Petit et al., 1999), resulting from feedbacks in the ocean, atmosphere and continental systems. Other important feedbacks include the thermohaline circulation, redistributing heat from the tropics to the poles, and the monsoon system, affecting global precipitation patterns (Huang et al., 2007). The complexity of the interplay between the different components of the climate system, including cause, response and feedback mechanisms, challenges predictions of how future climate will respond to on going addition of anthropogenic CO_2 to the atmosphere. This is why studying past climates may provide vital information on the integrated effect of the different feedbacks involved.

Our climate is currently changing rapidly due to a fairly new player in the climate system: human beings. Current anthropogenic greenhouse gas emissions have caused $p\text{CO}_2$ levels to exceed the range associated with glacial-interglacial cycles. Predictions are, that by the end of this century, atmospheric $p\text{CO}_2$ concentrations will range between 500 and 1000 ppm (parts per million), depending on the success of our efforts to reduce

emissions (IPCC, 2018; Rogelj, in press). In the likely case that we will not be able to reduce CO_2 emissions drastically, atmospheric concentrations may be as high as those found for the middle of the Eocene (40-50 million years ago), when temperatures were 5-8 °C higher (Sluijs et al., 2006; Zachos et al., 2006). This puts an increasing burden on next generations to accommodate negative carbon emissions (Hansen et al., 2016). At the same time, this emphasizes the need for accurate predictions of future climatic change, taking into account all potential feedbacks over both short and longer time scales. Besides reconstructing global average temperatures and atmospheric $p\text{CO}_2$ levels, a better understanding of other climate components is required as well, as these may be involved in tipping point behaviour of the climate system (Lenton et al., 2008; Rohling et al., 2012; Von der Heydt et al., 2016). For example, perturbations of the thermohaline circulation due to melt-water pulses are thought to have played an important role in rapid climate fluctuations, so-called Heinrich events, during the last glacial, while teleconnections played a major role in the so-called Dansgaard-Oeschger events (Bond et al., 1993; Cacho et al., 1999).

To better understand the processes behind such climate variability, $p\text{CO}_2$, temperature and seawater salinity need to be accurately reconstructed, using so-called proxies. Ideally, a proxy is affected by only one environmental parameter, and the original chemical signal should ideally not be altered after burial at the seafloor. These proxies include the composition of biochemical markers (e.g. bacterial or phytoplankton lipids), community composition of marine phytoplankton, air bubbles from ice cores, tree rings, fossil leaf stomata, etc. Also calcium carbonate fossils, accumulated on the seafloor, provide an often-used tool to reconstruct paleo-climates and -environments. The value of fossil shells and skeletons lies in their relatively good preservation potential, their global ubiquity and their geological longevity. The accuracy and precision of these reconstructions depends first and foremost on the robustness of the relationship between the environmental parameter (e.g. temperature) and the chemistry of the shell or skeleton.

Despite these uncertainties, biogenic calcium carbonate provides an invaluable source for paleo-reconstructions. One of the most widely used microfossils for this purpose are the shells of foraminifera, marine amoeboid protozoa. The majority of foraminifera produce a shell (often called a test) that is either agglutinated from collected particles, organic or calcareous. Species that produce calcareous shells are in the minority, but their paleoceanographic value exceeds that of the others as the chemistry of their shell holds valuable information. Moreover, calcifying foraminifera are responsible for 25-50% of the total open ocean carbonate production (Schiebel, 2002) and thereby play a major role in the oceanic carbon cycle.

The recognition of their proxy potential followed already shortly after the first description of foraminifera from beach sands by d'Orbigny (1826). The Challenger expedition (1873-1876) confirmed their ubiquity in the water column and seafloor sediments and started their first use for paleo-reconstructions (Cushman, 1918; Cushman and Wickenden, 1929). This was followed by the use of the chemical composition of their shells, after analytical developments in the 1940s and 1950's. Pioneering work of Urey (Urey, 1947; Urey et al., 1951) revealed the potential of stable oxygen isotope signals of foraminiferal shells as recorders of seawater temperature and global ice volume. This was extended by Emiliani (Emiliani, 1954, 1955) who was the first to study foraminiferal oxygen isotope records of sediment cores, which showed warmer and colder phases, providing the first empirical support of Milankovitch theory on orbitally paced glacial-interglacial cycles.

The success of these $\delta^{18}\text{O}$ -based paleo-reconstructions also fuelled the use of other proxies based on foraminiferal shell chemistry to reconstruct past climates (Fig. 1). Especially Mg/Ca is nowadays widely used for reconstructing seawater temperature (e.g. Nürnberg et al., 1996), while proxies based on other elements are still emerging. For instance Na/Ca has been suggested as a proxy for reconstructing past salinity (Allen et al., 2016; Mezger et al., 2018; Mezger et al., 2016), but is still accompanied by offsets and variability which is not fully understood. To further aid the development of proxies based on foraminiferal calcite needed for more accurate climate reconstructions, this thesis aims at a better understanding of foraminiferal trace element incorporation. Therefore I aim at i) linking environmental variables to shell chemistry, ii) describe element variability at the Nano scale of the calcite shell and iii) investigate foraminiferal control on element incorporation.

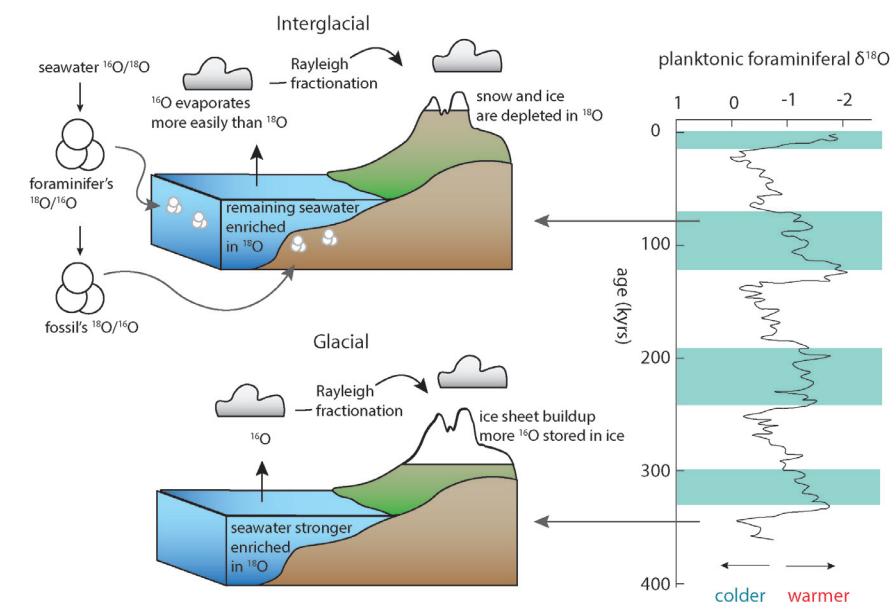


Figure 1. Foraminifera as paleoceanographic tools. When foraminifera produce their shells, they record the ratio of oxygen isotopes from the seawater and this signal is preserved after their burial in the seafloor (upper left corner). The oxygen isotopic composition of the seawater is in turn, determined by the amount of water stored in ice sheets (here represented by the snow-capped mountains) since water molecules with the lighter oxygen isotope (^{16}O) evaporate more easily than the heavier oxygen isotope (^{18}O), and more importantly, ^{18}O rains out more easily than ^{16}O during transport towards the poles: this process is called Rayleigh fractionation. Foraminifera producing their shells during interglacial periods (upper panel), when ice sheets are small, will have a relatively depleted oxygen isotopic composition (i.e. less ^{16}O , expressed usually as $\delta^{18}\text{O}$). In contrast, during glacial periods (lower panel), seawater is enriched in ^{18}O and foraminiferal shells have a higher $^{18}\text{O}/^{16}\text{O}$, due to the so-called glacial effect on $\delta^{18}\text{O}_{\text{seawater}}$. Additionally, temperature results in isotope fractionation during calcification, whereby warmer temperatures result in a more depleted $\delta^{18}\text{O}_{\text{calcite}}$. Sedimentary records that span glacial-interglacial cycles (right panel) hence show cyclicity in foraminiferal oxygen isotopic composition, which essentially reflect the alternation between colder and warmer periods. These alternations in foraminiferal isotope ratios are grouped into so-called Marine Isotope stages (MIS) of which the even numbers reflect glacials and odd numbers are interglacials. The planktonic foraminiferal $\delta^{18}\text{O}$ record here presented is based on Schmidt et al., 2006.

1.2 Brief introduction to foraminifera

Foraminifera are marine zooplankton and form a phylum within the Rhizaria (Cavalier-Smith, 2002). Their unique feature is the ability to extend their cell cytoplasm in the form of reticulate, granular pseudopodia (hence the alternative name for foraminifera: the Granuloreticulosa). The pseudopodia are used for locomotion, to collect particles

(Bowser and Travis, 2002) and to capture prey, including brine shrimps (Gupta et al., 2007) and diatoms (Austin et al., 2005). Many planktonic species have spines, thin elongated mono-crystal features, which are thought to play a role in guiding the cytoplasm and serve to keep the extracellular symbionts in place (figure 2). Many foraminiferal species harbour symbionts, mostly dinoflagellates, which move into the shell at night and stay within the rhizopodia and spines during the day (Hemleben et al., 2012). Foraminifera reproduce either sexually, by expelling gametes, or asexually, by mitosis (Stouff et al., 1999). The new cell immediately forms the first calcite chamber and then grows by the addition of consecutive chambers (figure 2). The chambers are interconnected and are occupied by the growing cell. The arrangement of the chambers, their ornamentation and the position and shape of the central opening (the aperture) are defining characteristics on which taxonomy (of fossil species) is based. Diversity of extant benthic foraminifera exceeds 2000 described species (Murray, 2007), which is in sharp contrast to the number of recent planktonic species. Until recently, there were ~50 species recognized (Aurahs et al., 2009), although recent molecular studies have questioned the traditional, morphological species concept for planktonic foraminifera (e.g. Morard et al., 2019). For extinct species, however, this morphology-based taxonomy is the only way to study evolution and ecological successions in past oceans. Changes in foraminiferal community composition are used for the reconstruction of past climates and environments, as species abundances reflect environmental preferences and hence conditions. For example, the recognition that benthic species reflect oxygenation and nutrient availability (Jorissen, 1999) allows for their qualitative reconstruction using changes in fossil species' abundances (e.g. Van der Zwaan et al., 1999). Furthermore, more quantitative reconstructions of past environmental variables can be derived from the chemical composition of foraminiferal shells.

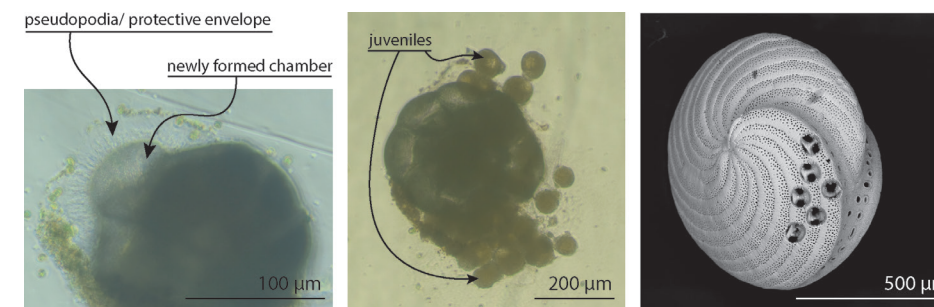


Figure 2. Calcification in foraminifera. Left: chamber addition by a living specimen of *Ammonia tepida* showing the primary organic sheet being calcified, surrounded by a dense network of pseudopodia. These pseudopodia form the so-called protective envelope that creates a microscopic environment in which the chemistry is controlled by the foraminifer (see also chapter 4 and synthesis of this thesis). Middle: an asexual reproduction event by a specimen of the same species in the JAMSTEC laboratory. Asexual reproduction is achieved by mitosis and results in the simultaneous release of tens and sometimes hundreds of one-chambered juveniles. Right: newly formed chambers of *Amphistegina lessonii* that are produced under controlled, experimental conditions (chapter 2). The holes are the result of laser ablation-ICP-MS measurements that were executed to determine the elemental composition (e.g. Na/Ca) of the newly formed chambers (image courtesy: Inge van Dijk).

1.3 Calcite chemistry of foraminiferal shells

With the exception of one genus, all extant calcifying foraminifera produce shells of calcite, using, potentially species-specific, biomineralization mechanisms. This is reflected in a wide variety of shell chemical compositions. The Ca^{2+} and inorganic carbon necessary to precipitate the calcite is thereby thought to be collected and concentrated from seawater in a confined calcifying space by the foraminifer (Toyofuku et al., 2017). During this process, which is so far only partly understood, other ions present in seawater are also incorporated in the foraminiferal shell, albeit in relatively low concentrations. Still, incorporation of a suite of trace elements has been shown to reflect seawater conditions. Ideally, these relationships are based on thermodynamic and kinetic laws that apply to inorganic calcites, yet in reality these relationships are strongly impacted by the biological control on shell formation.

The most studied trace element in foraminiferal calcite is Mg, expressed usually as its ratio to calcium (Mg/Ca), which shows an exponential relationship to seawater temperature in both inorganic calcites and in foraminiferal shells (Nürnberg et al., 1996) and is therefore widely applied to estimate past seawater temperatures (Billups and Schrag, 2002; Lear et al., 2000; Lear et al., 2002). Other foraminiferal proxies based on trace element incorporation include Ba as a tracer for salinity (Weldeab et al., 2007;

Weldeab et al., 2014), U as a proxy for carbonate chemistry (Keul et al., 2013b; Russell et al., 2004), Mn reflecting sea and or pore-water Mn concentrations (Klinkhammer et al., 2009; Koho et al., 2015), etc.. The amount of incorporated Na has been recently suggested as a novel proxy for seawater salinity (Mezger et al., 2016; Wit et al., 2013), but like for all proposed and applied proxies, potential pitfalls, e.g. the effect of other parameters, unknown lattice position, effects of diagenetic alteration and preservation on primary signals, hamper applicability. Understanding how the biomineralization pathway affects element incorporation may allow to better account for such pitfalls.

As an effect of organismal control, foraminiferal calcite chemistry (both element concentrations and isotope ratios) varies greatly between species (e.g. Bentov and Erez, 2006; Van Dijk et al., 2017b), specimens (e.g. Diz et al., 2012), chambers within individual shells (e.g. De Nooijer et al., 2014), and even within cross-sections of the shell wall (e.g. Hathorne et al., 2009; Kunioka et al., 2006). This variability, moreover, differs between elements: the concentration of magnesium for example, can vary up to a factor of ~100 between benthic species (Bentov and Erez, 2006) and ~5 between specimens and chambers (De Nooijer et al., 2014a), whereas Sr/Ca is shown in the latter study to vary less between species and is remarkable stable between individuals, within a factor of ~2%. These results indicate that individual elements are differently controlled by the organism.

For the use of foraminifera as paleoceanographic tools, inter-species variability in El/Ca (element/Ca) means that species-specific calibrations are required. Extinct species pose a challenge in this respect and reliable reconstructions hence can only be obtained if there is a solid relationship between taxonomy and calcite chemistry. To some extent, this is the case (De Nooijer et al., 2017; Van Dijk et al., 2017b), although closely related species may still vary considerably in their geochemistry. Sadekov et al. (2016) for example, showed that oxygen isotopes and Mg/Ca vary significantly between cryptic genotypes within the same species. Elements that are relatively variable between species appear to also be highly variable within chamber walls. Most notably, the heterogeneous distribution of Mg, with bands of higher and lower Mg concentrations, within the shell wall has been puzzling researchers over the last decade (Fig. 3, Fehrenbacher et al., 2017; Jonkers et al., 2016; Kunioka et al., 2006; Spero et al., 2015). This so-called element banding is also reported for B (Branson et al., 2015), Na (Branson et al., 2016) and S (Van Dijk et al., 2017a). In benthic foraminifera, banding appears to be associated with the location of the organic linings and hence sequential calcite addition (Kunioka et al. 2006, van Dijk et al. 2017a). In planktonic foraminifera the banding pattern appears to be related to circadian rhythm (Fehrenbacher et al., 2017), which possibly is also linked to episodes of calcite addition. Heterogeneity in element concentrations within the shell wall may also be caused by

diagenetic alteration of the shell chemistry in the sediment after burial. The outer layer of the shell that is in direct contact with the surrounding (pore) water can be re-mineralized, leading to high element concentrations of e.g. Mn (Hasenfratz et al., 2017; Yu et al., 2007). To account for any variability in calcite chemistry at all these scales, understanding the mechanisms behind element incorporation and preservation enhances proxy reliability.

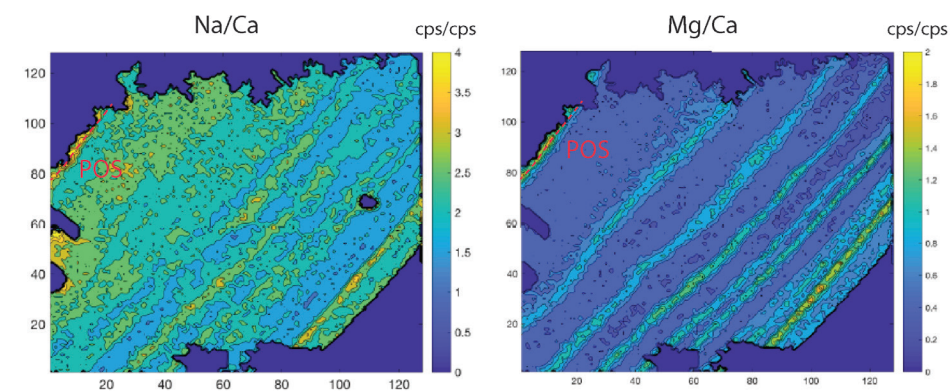


Figure 3. Distribution of Na and Mg (expressed as their ratio with respect to calcium) within a single chamber wall of the benthic foraminifer *Amphistegina lessonii*. Concentrations of both of these elements, as well as others (see chapter 3 for a more elaborate description) are elevated in so-called element bands. The bands with higher concentrations are often found to coincide with the position of the organic lining(s) within the shell wall. The position of the first organic lining that the foraminifer produces is indicated here in red (i.e. the primary organic sheet or POS). Electron Probe Micro Analysis maps have a width of 10 μm .

1.4 Biomineralization and element incorporation

Although surface seawater is oversaturated with respect to calcium carbonate, CaCO_3 does not spontaneously nucleate due to the presence of inhibiting ions, most notably Mg^{2+} , SO_4^{2-} and PO_4^{2-} (Morse et al., 2007). Foraminifera (as well as other marine calcifiers) have to overcome this inhibition either by increasing the saturation state or by selective removal of these inhibiting ions from the calcifying space (Zeebe and Sanyal, 2002). The low Mg/Ca values (1-10 mmol/mol) of many species suggest that they adopt the latter strategy, although other species are able to precipitate their calcite in the presence of relatively high concentrations of Mg (e.g. miliolid foraminifera: Toyofuku et al., 2000; Van Dijk et al., 2017b). Besides magnesium, the partitioning of other elements and fractionation of isotopes indicate that foraminifera actively control the chemistry of the fluid from which they precipitate their calcite (figure 4).

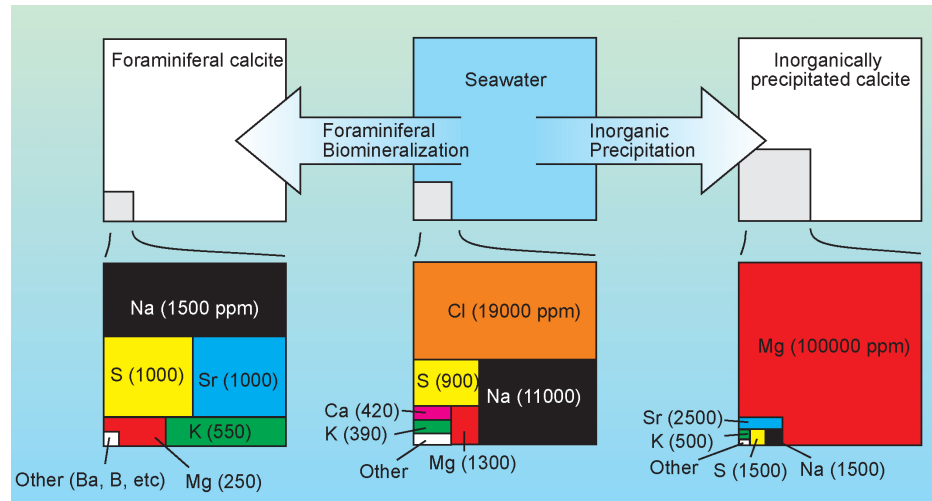


Figure 4. Foraminiferal calcium carbonate (left) has a markedly different element composition that CaCO_3 precipitated inorganically from seawater (right). From De Nooijer et al. (2014b).

Various micro- and Nano-scale observation techniques have identified a number of key components of foraminiferal chamber formation. Earlier studies using (transmission electron) microscopy showed that foraminifera start the formation of a new chamber wall with the production of a primary organic sheet (or POS; also termed Anlage or primary organic membrane; Angell, 1979; Banner et al., 1973; Bé et al., 1979; Hemleben et al., 1986; Spero, 1988). This organic layer serves as a template on which the first calcite precipitates and also defines the overall shape of the new chamber. At the same time, these studies showed that the POS and later, the growing calcite surface was separated from the seawater surrounding the foraminifer by a so-called protective envelope (the PE; Angell, 1979; Spindler, 1978), that secludes a microscopic zone in which the calcite precipitates: the site of calcification (SOC). This SOC is analogue to the calcifying fluid in corals (e.g. Sinclair and Risk, 2006) or the calcifying vesicle in coccolithophores (e.g. Brownlee et al., 2015). More recent studies have identified different stages and the involvement of actin meshworks in the formation of the outer protective envelope (Nagai et al., 2018a; Tyszkla et al., 2019), showing the mechanism by which foraminifera are able to separate the site of calcification from the surrounding seawater. The dimensions and shape of the SOC is also quantified by combining SEM with Focussed Ion Beam (FIB) on fixated calcifying foraminifera (Nagai et al., 2018b). These studies are important in identifying the biochemical nature of the protective envelope and thereby constrain the mechanism by which ions enter the SOC (e.g. De Nooijer et al., 2014b, Fig. 5).

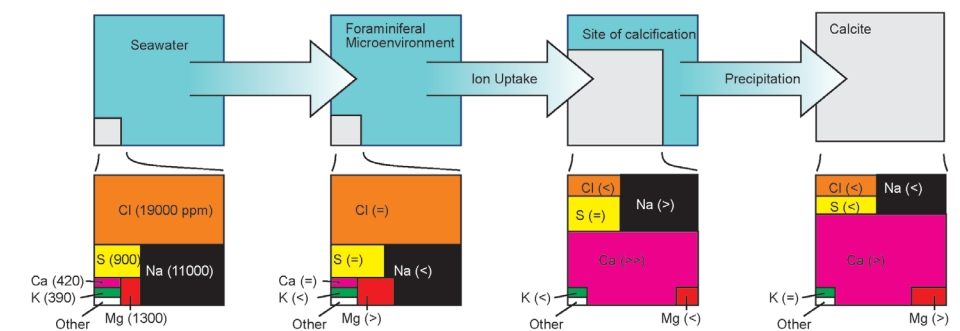


Figure 5. The different steps in foraminiferal calcification. The composition of seawater may be altered by biological activity of the foraminifer (and its symbionts) so that the seawater in the direct vicinity of the foraminifer, serving as the basis for calcification, is different from that of ambient seawater. Uptake of ions from the environment further alters the composition (middle panels) after which precipitation of CaCO_3 at the site of calcification add to the overall fractionation (right). In this example, only (major) elements are depicted, but fractionation of stable isotope ratios is subject to the same processes.

If the outer protective envelope is not fully sealing the SOC from the environment, ions for calcification could be delivered through seawater exchange to the SOC. If, however, the PE would completely shield the SOC from the environment, all ions must be transported over the PE's membranes. There are indications and good arguments for both these options: chapter 3 and the outlook of this thesis aim at reconciling these hypotheses and explain trace element incorporation as a function of both an open and closed SOC.

In the small, symbiont-barren benthic foraminifers *Ammonia* and *Cibicides*, calcification is accompanied by a strong, outward proton pumping (De Nooijer et al., 2009b; Glas et al., 2012; Toyofuku et al., 2017). This results in a low pH directly outside of the PE, and likely a high pH inside the SOC, which is suggested to drive an inward flux of CO_2 (Toyofuku et al., 2017). Stable isotope spiking experiments using *Ammonia* showed that at the same time, calcium ions are being transported inward (Nehrke et al., 2013). It is highly likely that the outward H^+ pumping and inward Ca^{2+} flux are directly coupled and that this exchange of ions drives the creation of a highly supersaturated fluid from which calcite precipitates. It may be that the Ca^{2+} transport mechanism occasionally transports other ions and hence Mg^{2+} , Ba^{2+} , etc. are incorporated. Alternatively, the conditions in the SOC may determine the partitioning and hence eventual El/Ca , for example through kinetic effects set by the calcite precipitation rate. Calcium pumping rates may differ between species, for example, resulting in differences in the SOC's saturation state and hence, precipitation rates. Since higher rates are known to enhance Mg incorporation (e.g. Burton and Walter, 1991), such differences may also explain differences in foraminiferal Mg/Ca .

Over the last ~2 decades, various (conceptual and numerical) models have been proposed to explain observations on element incorporation and isotope fractionation in foraminiferal calcite. A pioneering biomineralization model for element incorporation has been proposed by Elderfield et al. (1996) and includes precipitation from a calcifying fluid, similar to that proposed for corals (Sinclair and Risk, 2006). This model calculates the El/Ca ratio from CaCO_3 precipitation from a confined space using published inorganic equilibrium partition coefficients. As these are low for elements that fit relatively poorly in the calcite lattice, the concentrations of these elements will increase in the confined space while Ca is being depleted, resulting in more incorporation of these elements towards the end of chamber formation. This mode of element incorporation is proposed to be additionally determined by replenishment of the confined space with seawater in between chamber addition. In reality, this is questionable since the total amount of calcium ions for the production of a new chamber, would require a volume of >50 times the volume of the foraminifer; De Nooijer et al., 2009). However, if Ca is constantly being pumped into the SOC from the surrounding environment, this would dilute the trace element concentration in the SOC gradually, and, depending on element partitioning coefficients and the pumping rate this may counteract Rayleigh fractionation. Therefore, Nehrke et al. (2013), proposed the so-called trans-membrane transport (or TMT) model, with active transport of Ca into the SOC and occasional, possibly non-selective, transport of other ions that determine the El/Ca of the SOC. In this model, there is the possibility that seawater enters the SOC occasionally and serves as an additional source of ions. This was suggested in a conceptual model proposed by Bentov and Erez (2006) and was based on observations on calcifying specimens of *Amphistegina lessonii*. This model also assumes active Mg removal from these vesicles to lower Mg concentrations of the calcite and hence explains the relatively low partition coefficient for Mg in foraminiferal calcite.

Additional controls that determine foraminiferal calcite chemistry include i) adsorption of elements to the organic template and ii) phase transformations. Firstly, the organic compounds that constitute the primary organic sheet may selectively bind ions from the SOC as well as influence crystal morphology and thereby determine the overall El/Ca and within-wall element banding. Particularly the charged groups of amino acids within the POS are hypothesized to adsorb positively charged ions differently (Branson et al., 2016). This role of the organic lining may help explaining the high concentrations of cations in the vicinity of the POS (e.g. Branson et al., 2015; Kunioka et al., 2006). Secondly, whether calcium carbonate forms by monomer-monomer attachment or by crystallization from an (amorphous) precursor is important for the partitioning of elements (e.g. De Yoreo et al., 2015). Recently it was shown that foraminiferal calcite is transformed from vaterite (Jacob et al., 2017), which complicates comparison of foraminiferal calcite chemistry

with many inorganically precipitated calcites since the latter often follow the kink-step-mode of precipitation (Morse et al., 2007).

1.5 Synopsis

Many elements and stable isotopes are incorporated with different partition coefficients, and fractionated differently, among species and with different sensitivities to environmental parameters. This has been documented for Mg (e.g. Sadekov et al., 2016; Wit et al., 2012), $\delta^{18}\text{O}$ (e.g. Toyofuku et al., 2011) and $\delta^{11}\text{B}$ (e.g. Sanyal et al., 2001), but not yet for other proxies. Na/Ca is an emerging salinity proxy (Wit et al., 2013; Allen et al., 2016; Mezger et al., 2016), but incorporation of sodium is so far unrecorded in species precipitating intermediate- or high-Mg calcite. In **Chapter 2**, we present results from a culturing study with *Ammonia tepida* and *Amphistegina lessonii*. The temperate *A. tepida* precipitates calcite with a relatively low Mg content (2-5 mmol Mg/mol Ca), while the symbiont-bearing, tropical *A. lessonii* has a shell with approximately 10 times more Mg. This difference is thought to reflect differences in biomineralization controls and may well affect the incorporation of other elements likewise. This culturing experiment was also conducted to expand the salinity range over which Na/Ca is reported to respond to salinity.

Between salinities 20 and 40, Na/Ca-salinity sensitivity is surprisingly similar, with an increase of 0.064 (for *A. tepida*) and 0.077 mmol/mol (for *A. lessonii*) increase per salinity unit. This is despite the difference in absolute Na/Ca, which is approximately 2-fold higher in *A. lessonii* than in *A. tepida* (~10 mmol/mol and 5 mmol/mol, respectively). In addition, electron microprobe mapping of incorporated Na and Mg of the cultured specimens shows that within chamber walls of *A. lessonii*, Na and Mg occur in bands of elevated concentrations in close proximity of the primary organic sheet. Between species, Mg banding is relatively similar, even though Mg content is 10 times lower and that variation within the chamber wall is much less pronounced in *A. tepida*. In addition, Na banding is much less prominent in this species than it is in *A. lessonii*. Inter-species differences in element banding reported here are hypothesized to be caused by differences in biomineralization controls responsible for element uptake.

In **Chapter 3**, the characterization of element banding within chamber walls is further elaborated upon. To understand the cause of the, by now well-documented banding of elements within foraminiferal chamber walls, it is necessary to determine peak- and trough heights (i.e. concentrations), their widths and the position of these high-concentration bands in relation to the (primary) organic sheet. Since specimens within one species are known to have different El/Ca ratios (e.g. De Nooijer et al., 2014a), characterization of

the high- and low element banding may allow explaining inter-specimen differences in average El/Ca. Moreover, comparing species may also hint towards an explanation for the inter-species differences in average element/ Ca (e.g. chapter 2 of this thesis).

Specimens of the low-Mg species *Ammonia tepida* and of the intermediate-Mg species *Amphistegina lessonii* were grown at different temperatures and salinities to assess how these parameters influence the element concentrations within the shell wall. Embedded and polished specimens were analysed by NanoSIMS to evaluate the distribution of a suite of elements. Results show that Mg, Na, Sr and K are co-located within shells, and occur in bands that coincide with organic linings but extend further into the calcite lamella. Changes in temperature or salinity modulate the element-banding pattern as a whole, with peak and trough heights co-varying rather than independently affected by these two environmental parameters. This means that independent changes in peak or trough height do not explain differences in average El/Ca between specimens. These results are used to evaluate and synthesize models of underlying mechanisms responsible for trace and minor element partitioning during calcification in foraminifera.

Observations on biomineralizing foraminifera show the involvement of seawater vacuoles, pH-regulation, organic templates, etc. These structures and processes determine the conditions and element composition of the calcifying fluid from which foraminifera precipitate their calcite. Within this confined space, kinetic and thermodynamic effects determine element partitioning and hence the eventual composition of the calcite shell. Environmental conditions (e.g. temperature or salinity) likely modulate these effects and hence element incorporation, but may also influence the biological controls on calcite precipitation. Although models for foraminiferal biomineralization are able to explain part of the observed sensitivities of element incorporation to environmental parameters, the largest unknown with a profound impact on foraminiferal shell chemistry, remains the calcite crystal precipitation rate.

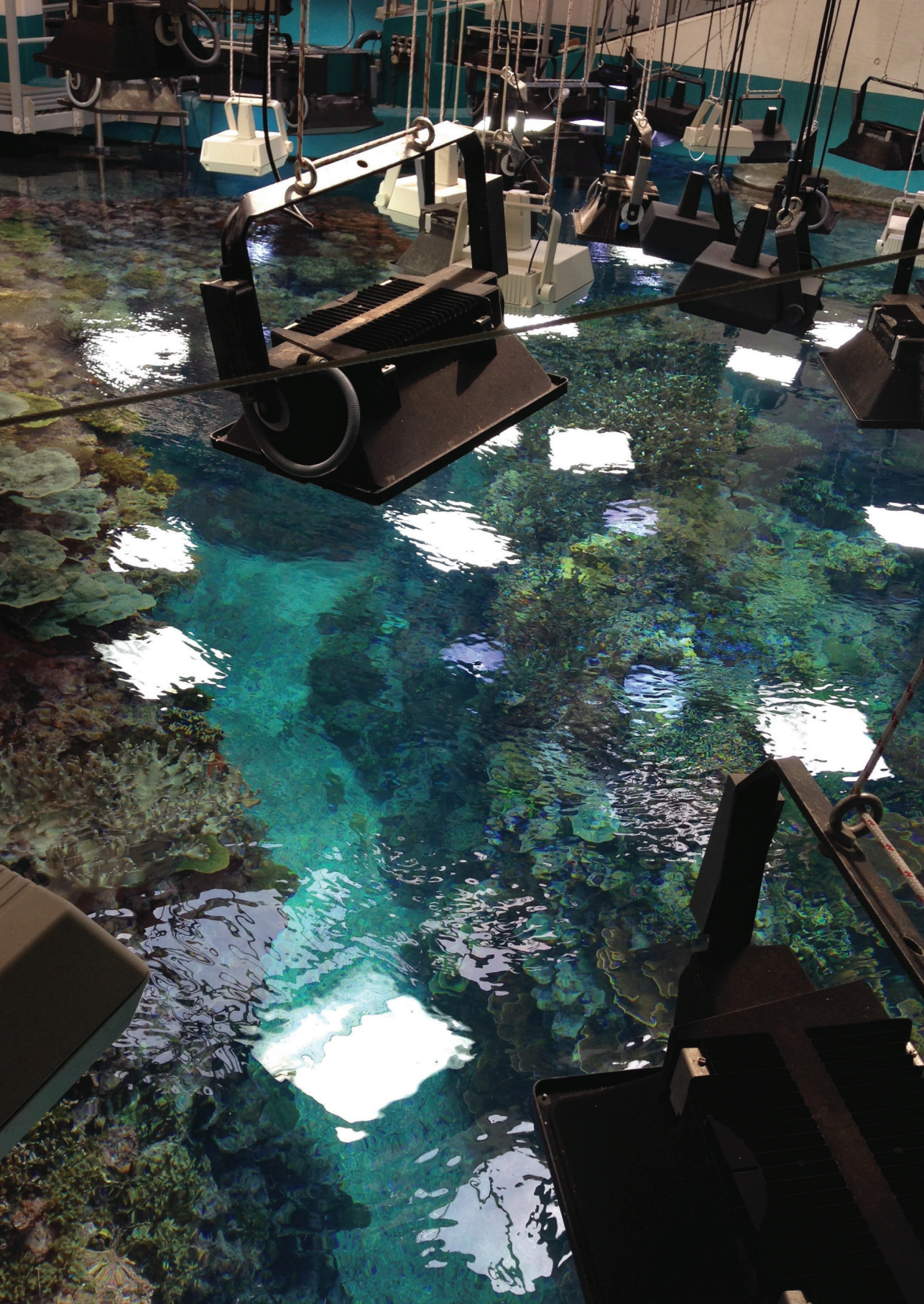
Chapter 4 describes labelling experiments in which the incorporation of Sr-spikes during calcification were visualized using NanoSIMS to determine precipitation rates in the foraminifer *A. tepida* throughout chamber formation. Results show that precipitation rates are surprisingly uniform between specimens at $\sim 22 \pm 2$ nmol/cm²/min, a rate comparable to the fastest rates obtained by inorganic calcite precipitation experiments. We show that the amount of Sr incorporated in foraminifera can be explained as a function of precipitation rate in a Mg-free, seawater-like solution. In addition, the precipitation rate is significantly lower in the first layer of calcite, compared to the rest of the shell wall, which may be explained by the presence of inhibitors like

magnesium- and sulphate ions at the onset of calcification. This suggests that Ca and Sr are directly transported to the calcifying fluid, whereas Mg and possibly other ions such as S and Na, cannot reach the calcifying fluid after calcification has started. These results aid the understanding of the role of precipitation rate (i.e. kinetics) on trace element partitioning and element banding in foraminifera and thereby, improve their reliability as paleoceanographic reconstruction tools.

Orbitally controlled hydrological changes in the Eastern Mediterranean display a complex interplay of different latitudinal climate processes. This hydrological variability, in concert with increased organic matter production, has led to regular seafloor anoxia and the deposition of so-called 'sapropels', dark-organic rich layers in the marine sediment record. However, the exact triggers of sapropel onset and termination remain debated, whereby the relative influence of Nile outflow and a concomitant decrease in surface salinity as well as changes in seawater temperature may have enhanced stratification.

In **Chapter 5** we apply novel proxies based on element incorporation into foraminiferal calcite to compare sea surface temperature and salinity variability for ten sapropels in the Eastern Mediterranean over the past 350 kyr. Estimates of Nile river outflow (Ba/Ca, Mn/Ca), surface salinity (excess-Mg/Ca, Na/Ca) and $\delta^{18}\text{O}_{\text{water}}$ are compared to established sediment geochemistry proxies recording monsoon strength (Ti/Al), productivity (Ba/Al) and bottom water deoxygenation (Mo/Al). Our results show that both changes in temperature and Nile-river runoff play a (consistent) role in sapropel onsets and terminations, albeit with variable amplitudes for the different sapropels. Throughout this thesis we have observed variability in foraminiferal El/Ca at multiple scales, linked to environmental variables such as salinity and temperature, and due to biomineralization processes, resulting in inter-species and -specimens variability and element banding within the shell wall. Linking this variability mechanistically to the process of biomineralization is needed for a more robust application of proxies.

In **Chapter 6** we aim to synthesize observations on biomineralization and trace element uptake by developing a numerical model for element incorporation as a function of wall thickness. The model combines and extends previously proposed models and is based on calcite precipitation from a closed biomineralization reservoir combined with trans-membrane Ca^{2+} -transport. Model parameters include element concentrations in seawater, the volume of the biomineralization reservoir and inorganic partition coefficients, which are based on observations, and assumptions for the Ca^{2+} -transport. Our model explains differences between element signatures between species by a difference in reservoir volume (i.e. the SOC), and furthermore explains element banding within the shell wall.



Chapter 2

Impact of salinity on element incorporation in two benthic foraminiferal species with contrasting Magnesium contents

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Abstract

Accurate reconstructions of seawater salinity could provide valuable constraints for studying past ocean circulation, the hydrological cycle and sea level change. Controlled growth experiments and field studies have shown the potential of foraminiferal Na/Ca as a direct salinity proxy. Incorporation of minor and trace elements in foraminiferal shell carbonate varies, however, greatly between species and hence extrapolating calibrations to other species needs validation by additional (culturing) studies. Salinity is also known to impact other foraminiferal carbonate-based proxies, such as Mg/Ca for temperature and Sr/Ca for seawater carbonate chemistry. Better constraints on the role of salinity on these proxies will therefore improve their reliability. Using a controlled growth experiment spanning a salinity range of 20 units and analysis of element composition on single chambers using laser ablation-Q-ICP-MS, we here show that Na/Ca correlates positively with salinity in two benthic foraminiferal species (*Ammonia tepida* and *Amphistegina lessonii*). The Na/Ca values differ between the two species, with an approximately 2-fold higher Na/Ca in *A. lessonii* than in *A. tepida*, coinciding with an offset in their Mg content (~35 mmol/mol versus ~2.5 mmol/mol for *A. lessonii* and *A. tepida*, respectively). Despite the offset in average Na/Ca values, the slopes of the Na/Ca-salinity regressions are similar between these two species (0.077 versus 0.064 mmol/mol change per salinity unit). In addition, Mg/Ca and Sr/Ca are positively correlated with salinity in cultured *A. tepida*, but show no correlation with salinity for *A. lessonii*. Electron microprobe mapping of incorporated Na and Mg of the cultured specimens shows that within chamber walls of *A. lessonii*, Na/Ca and Mg/Ca occur in elevated bands in close proximity to the primary organic lining. Between species, Mg-banding is relatively similar, albeit that Mg content is 10 times lower and that variation within the chamber wall is much less pronounced in *A. tepida*. In addition, Na-banding is much less prominent in this species than it is in *A. lessonii*. Inter-species differences in element banding reported here are hypothesized to be caused by differences in biomineralization controls responsible for element uptake.

2.1 Introduction

Seawater salinity varies over time and space as a function of continental ice volume, evaporation, precipitation and river runoff. Salinity reconstructions could provide important constraints on past ocean circulation, the hydrological cycle and glacial-interglacial sea level changes. Currently, most reconstructions of salinity are indirect and based on the correlation between salinity and $\delta^{18}\text{O}_{\text{water}}$, assuming this relationship to be constant over space and time (Rohling and Bigg, 1998). An independent salinity proxy may reduce the uncertainties inherently associated with such approaches (Rohling and Hilgen, 2007) and should preferably be based on one of the main components of seawater salinity, for instance sodium (Na). Results from a culture study showed that the sodium content of foraminiferal calcite (Na/Ca_{cc}) correlates positively and linearly with salinity for the benthic low-Mg, symbiont-barren species *Ammonia tepida*, with a sensitivity of 0.22 mmol/mol for every change of 1 salinity unit between salinities 30 and 38.6 (Wit et al., 2013). Various culture studies earlier showed that also Mg/Ca is affected by salinity, but responds more strongly to changes in temperature (Dissard et al., 2010b; Hönisch et al., 2013; Lea et al., 1999; Nürnberg et al., 1996). Although an effect of salinity on foraminiferal Sr/Ca_{cc} has been reported in some studies (Dissard et al., 2010b; Kısakürek et al., 2008; Wit et al., 2013) other studies did not find a relation between salinity and foraminiferal Sr/Ca (Allen et al., 2016; Diz et al., 2012; Dueñas-Bohórquez et al., 2009), which lead to the hypothesis that foraminiferal Sr/Ca mainly reflects seawater inorganic carbon chemistry (Keul et al., 2017) in addition to its response to temperature (Lea et al., 1999; Raja et al., 2007). Hence, an independent salinity proxy would not only be useful for constraining past (changes in) salinity, but also improve temperature reconstructions based on Mg/Ca_{cc} and reconstructions of past seawater carbonate chemistry based on Sr/Ca.

Following the culture-based Na/Ca_{cc}-salinity calibration for *A. tepida* (Wit et al., 2013), a culture study with planktonic symbiont-bearing species also showed a significant linear relationship for *Globigerinoides ruber* (Allen et al., 2016). Although no significant relationship was observed in this study for *G. sacculifer* (Allen et al., 2016), a recent field calibration observed positive linear relationships for both these species (Mezger et al., 2016). Still, the Na/Ca-salinity sensitivities observed between the different species and studies differed considerably (ranging from a change in 0.074 to 0.66 mmol/mol in Na/Ca_{cc} for a change in 1 salinity unit). Whereas Wit et al. (2013) suggested an incorporation mechanism similar to that observed in inorganic calcite, field and culture studies also show that different species of foraminifera have varying calcite chemistries, thereby resulting in the need of species-specific calibrations similar to many other foraminiferal

trace metal-based proxies (e.g. Anand et al., 2003; Bemis et al., 1998; Elderfield and Ganssen, 2000; Rosenthal et al., 2000; Toyofuku et al., 2011). For example, Mg/Ca_{cc} values are different between groups of low-Mg-, high-Mg hyaline and porcelaneous foraminifera (Raja et al., 2007; Segev and Erez, 2006; Toyofuku et al., 2000), which also seems to be reflected in other co-precipitated cations (De Nooijer et al., 2017). Hence, calibration of Na/Ca_{cc} as a function of salinity for other species is not only necessary to test the applicability of this novel proxy for other groups of foraminifera, but also allows testing whether monovalent cations follow the inter-species trends described for divalent cations (Terakado et al., 2010).

Here we calibrated Na-, Mg- and Sr-incorporation in the intermediate-Mg calcite benthic foraminiferal species *Amphistegina lessonii* and the low-Mg calcite species *Ammonia tepida* over a salinity range of 20 units (from 25 to 45). We thus compare the El/Ca versus salinity trends in a tropical, symbiont-bearing species (*A. lessonii*) to a temperate intertidal symbiont-barren species (*A. tepida*) and both of them to existing calibrations. The chemical composition of newly formed calcite was determined by Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-Q-ICP-MS), providing insights in concentrations and variability therein between specimens and between single chambers. To investigate intra-specimen variability at the scale of the chamber wall we also performed Electron Probe Micro Analysis (EPMA), mapping the Ca, Na and Mg distribution throughout the chamber wall for specimens of both species cultured.

2.2 Methods

2.2.1 Culture media preparation and chemistry

In total, 50 L of seawater with a salinity of 50 was prepared by sub-boiling 0.2 μm filtered North Atlantic seawater for 48 hours at 45 °C. Subsequently, culture media were obtained by diluting this high-saline seawater with double de-ionized seawater (~18 M Ω) in batches of approximately 10L with salinity increasing from 25 to 45 in steps of 5 units, resulting in 5 unique salinity conditions. Using a single batch of concentrated seawater to subsequently dilute to the desired salinities ensures constant element to Ca ratios. Salinity of the media was measured with a salinometer (VWR CO310), based on conductivity. Culture media were stored in Nalgene containers and kept in the dark at 10 °C. Seawater pH was determined with a pH meter (pH110, VWR). Subsamples were taken prior to and at the end of the experiment and analyzed for DIC and element concentrations to monitor the effect of sub-boiling on the seawater's inorganic carbon chemistry and element composition (Table 1). Subsamples for DIC were collected in headspace-free vials and conserved with a saturated $HgCl_2$ solution (10 μl $HgCl_2$ /10 ml

sample). DIC measurements were performed on an autoanalyzer spectrometric system TRAACS 800; Stoll et al. (2001). This analysis requires only a small amount of sample, while yielding high accuracy (± 2 $\mu mol/kg$) and precision (± 1.5 $\mu mol/kg$). The minor and major elemental composition of the culture media was measured using a sector field ICP-MS (Element2, Thermo Scientific) by sampling 1 ml from the culture media and dilution by a factor 300 with 0.14 M HNO_3 (Table 1).

Table 1. Experiment culture media measurements per salinity condition. Carbonate ion concentrations and saturation state with respect to calcite (at 25 °C) were calculated using CO2SYS (Van Heuven et al., 2011) and the equilibrium constants K1 and K2 of Mehrbach et al. (1973), as reformulated by (Dickson and Millero, 1987).

Experiment	Na/Ca_{sw} mol/mol	Mg/Ca_{sw} mol/mol	Sr/Ca_{sw} mmol/mol	Salinity	DIC $\mu mol/kg$	pH	$[CO_3^{2-}]$ $\mu mol/kgSW$	$\Omega_{calcite}$
S25	48.84	5.61	9.37	25.2	1087.3	8.32	164.90	4.28
S30	49.79	5.69	9.45	30.3	1305.3	8.28	205.98	5.15
S35	48.56	5.51	9.04	35.2	1512.0	8.22	258.84	6.22
S40	48.50	5.62	9.19	40.0	1734.4	8.17	267.23	6.16
S45	48.90	5.73	9.21	45.2	1947.4	8.10	284.67	6.23

2.2.2 Collection of foraminifera and culture set-up

Surface sediment samples containing foraminifera (*A. lessonii*) were collected from the Indo-Pacific Coral Reef aquarium in Burgers' Zoo (Arnhem, The Netherlands; Ernst et al., 2011) and a tidal flat near Den Oever, the Wadden Sea (*A. tepida*, genotype T6; Hayward et al., 2004). Sediment was stored in aerated aquaria at 25°C (*A. lessonii*) and 10°C (*A. tepida*) with a day/night cycle of 12/12 hours, similar to conditions in the coral reef aquarium and Wadden Sea, respectively. From both stocks, living specimens, recognized by chambers that were filled with yellow cytoplasm and pseudopodial activity, were isolated.

The culture protocol was the same for both species to facilitate comparison of obtained Element/Ca ratios between species. Since our specimens of *A. tepida* are from a location with a much larger temperature range than where *A. lessonii* is derived from (De Nooijer et al., 2014a; Ernst et al., 2011; van Aken, 2008), both species were incubated at 25 °C. Living specimens were placed in groups of 25 individuals in Petri dishes with approximately 70 ml of North Atlantic surface seawater (0.2 μm filtered) and fed with fresh cells of the algae *Dunaliella salina*. After reproduction, which occurred in approximately 2/3 of all incubated specimens in both species, 2-3 chambered juveniles were isolated. The use of specimens from reproduction events guarantees that virtually

all chambers present at the end of the experiment were produced under the culture conditions (De Nooijer et al., 2014a). Strains of specimens of the reproduction events were divided over Petri dishes (resulting in 2-10 individuals per dish) with approximately 10 ml culture medium and stored in a temperature controlled incubator set at 25 °C with a day/night cycle of 12/12 hours. The culture media in the Petri dishes were replaced once every week, after which specimens were fed with 1 ml concentrated and freeze-dried *Dunaliella salina* diluted with the culture medium for each salinity condition, to minimize changes in salinity when feeding the foraminifers. The amount of food was adjusted so that it was not depleted after a week, at the same time not resulting in an excess of debris and hence reducing bacterial growth. Petri dishes were sealed with a lid to minimize evaporation. After 6-8 weeks, specimens were harvested and transferred to microvials to clean the specimens' carbonate shells from cell material. Specimens were cleaned with an adapted version of the Barker protocol (Barker et al., 2003), only applying the organic removal / oxidation step, in which NaOH was replaced with NH₄OH, in order to avoid Na-contamination of our samples. Organic matter was removed by adding 1% H₂O₂ buffered with 0.1M NH₄OH at 90 °C and gentle ultrasonication (80kHz, 50% power, in degas mode) for 1 min, which is known not to affect obtained Mg/Ca and Sr/Ca (Barker et al., 2003). Specimens were subsequently rinsed 3 times with double de-ionized water, dried in a laminar flow cabinet, after which their size was determined (i.e. the maximum diameter crossing the center of the specimen). The specimens were thereafter stored until geochemical analyses (LA-Q-ICP-MS; 2.2.2 and EPMA; 2.4).

2.2.3 Foraminiferal calcite chemistry

Specimens were fixed on a laser ablation-stub using double sided tape, carefully positioning them to allow ablation of the last chambers (Appendix A). Element concentrations of individual chambers were measured with LA-ICP-MS (Reichert et al., 2003). The last 1-3 chambers of each specimen were ablated using a circular spot with a diameter of 60 µm (*A. tepida*) and 80 µm (*A. lessonii*) (NWR193UC, New Wave Research) in a helium environment in a New Wave TV2 dual-volume cell (cup volume of ~1 cm³) at a repetition rate of 6 Hz and an energy density of approximately 1 J/cm². The aerosol was transported to a quadrupole ICP-MS (iCap, Thermo Scientific) on a helium flow at a rate of 0.7 L/min, with 0.4 L/min Argon make-up gas being added before entering the torch. Nitrogen gas was added at a rate of 5 ml/ minute to enhance sensitivity of the analysis. Before entering the torch, the aerosol/ Ar/ He mixture passed through an in-house made smoothing device to reduce temporal variations in signal strength. Monitored masses included ⁷Li, ¹¹B, ²³Na, ²⁴Mg, ²⁵Mg, ²⁷Al, ⁴³Ca, ⁴⁴Ca, ⁶⁰Ni, ⁶⁶Zn, ⁸⁸Sr, and ¹³⁷Ba, with one full cycle through the different masses taking 120 ms. Calibration was performed against a MACS-3 (synthetic calcium carbonate) pressed powder carbonate

standard with ⁴³Ca as an internal standard. Count rates for the different masses were directly translated into element/Ca_{cc} (El/Ca_{cc}) ratios. Internal precision based on MACS-3 is 4% for Na, 3% for Mg and 4% for Sr. Accuracy and relative analytical errors, based on measuring international standards JCp-1 coral (*Porites* sp.) powder and the NIST (National Institute of Standards and Technology) SRM 610 and SRM 612 (glass) are listed in Table 2. The relatively large offset between the glass standards and the pressed powders (both MACS-3 and JCp-1) is known not to influence obtained El/Ca_{cc} ratios when either one is used as calibration standard (Hathorne et al., 2008), but due to the similar matrix, MACS-3 was chosen as calibration standard.

Table 2. Accuracies (Ac) and precisions (Pr) for Na, Mg and Sr for the various standards analyzed.

Standard	n	Ac Na (%)	Pr Na (%)	Ac Mg (%)	Pr Mg (%)	Ac Sr (%)	Pr Sr (%)
JCp-1	51	99	6	96	6	96	4
NIST610	32	119	3	104	2	110	3
NIST612	29	119	3	104	2	110	2

In total, 675 chambers were measured (336 for *Amphistegina* and 339 for *Ammonia*), resulting in between 52 to 125 single chamber measurements per salinity condition per species. These measurements were done on the last three (final or F, penultimate or F-1 and pre-penultimate or F-2) chambers of these specimens (see Table 3 for number of specimens and average number of spots per specimen). Element concentrations were calculated from the time (i.e. ablation depth) resolved profiles using an adapted version of the SILLS (Signal Integration for Laboratory Laser Systems; Guillon et al., 2008) package for MATLAB (for details see Van Dijk et al., 2017a), while taking care to exclude contaminations potentially present on chamber walls (examples of profile selection: Duenas-Bohorquez et al., 2011a; Mewes et al., 2014; Mezger et al., 2016; Van Dijk et al., 2017c; Wit et al., 2013). Measurements with ablation yields or integrations times <5 s were excluded from further analysis.

The LA measurements were also used to investigate the co-occurrence of elements within specimens. Since there is variability in Ca counts between the laser ablation measurements, single-spot based Element/Ca_{cc} ratios may cause spurious correlation due to coupled differences in Ca counts. To test whether observed correlations between Na/Ca_{cc}, Sr/Ca_{cc} and Mg/Ca_{cc}, based on single-spots, are due to the use of a common denominator (Ca), we performed a Monte Carlo simulation. In short, the correlation coefficients between randomly drawn single-spot Mg concentration, divided by measured Ca, and measured Na/Ca_{cc} concentrations were compared to the correlation coefficient of measured Na/Ca_{cc} and Mg/Ca_{cc} concentration ratios in our dataset. By

using a Kernel fit of the measured data set to draw the random data set and using the measured Ca as a common denominator we effectively simulate the spurious correlation. The Monte Carlo results show that inter-element correlations are not spurious, since the measured correlation coefficient is significantly higher than the distribution of the correlation coefficients between 10,000 randomly drawn El^1 concentrations/measured Ca concentration and measured El^2 /Ca concentrations (Appendix B).

Furthermore, to test whether Sr/Ca_{cc} and Na/Ca_{cc} variability in *A. lessonii* is not caused by variability in Mg content due to a potential closed sum effect (since high amounts of incorporated Mg cations could reduce the Ca content of the shell and hence result in apparently elevated Sr/Ca_{cc} and Na/Ca_{cc}), we calculated maximum variability due to the sole effect of Mg-substitution. For *A. lessonii*, variability (standard deviation) of ± 0.09 mmol/mol in Na/Ca_{cc} and ± 0.016 mmol/mol in Sr/Ca_{cc} around the mean could be caused by variability in Mg/Ca_{cc} (assuming Mg substitutes for Ca in the calcite lattice, and Mg plus Ca approximates 1 mol per mol calcite). This may have influenced the Sr/Ca_{cc} and Na/Ca_{cc} regression slopes over salinity and also the calculated inter-element correlation coefficients, but only by a maximum of $\pm 1\%$ for both elements, which is considerably lower than the total observed variability within the dataset of 16% and 9%, respectively.

2.2.4 Electron Microprobe Mapping

To investigate variation of element distribution across the chamber wall, a number of cultured specimens were prepared for Electron Microprobe Analysis (EPMA). From each of the five salinity conditions, six specimens from both species were selected and embedded in resin (Araldite 2020) in an aluminum ring (diameter 1 cm) in a vacuum chamber. Samples were polished with a final polishing step using a diamond emulsion with grains of 0.04 μm . This procedure resulted in exposure of a cross-section of the foraminiferal chamber wall from which areas for EPMA mapping were selected (Appendix A). These areas were selected for being perpendicular to the shell outer surface, resulting in pores completely crossing the exposed chamber wall. Elemental distributions were mapped in chambers prior to F-3 to study the element distribution across the various layers of calcite (lamella) produced with the addition of each new chamber in rotaliid foraminifera (Reiss, 1957, 1960). Elemental distribution in the shell wall was measured using a field emission Electron Probe Micro Analyser (JEOL JXA-8530F HyperProbe) at 7.0kV with a dwell time of 350 ms, using a spot diameter of 80 nm and a step size between 0.1538 μm and 0.4072 μm (130 x 130 pixels).

Spatial resolution of the EPMA mapping was determined using the software package CASINO (monte CARlo SIMulation of electroN trajectory in SOLids, v 2.48). With the

input parameters identical as used in our analysis (80 nm spot size, beam current 7 KeV, etc.), the simulated surface radius of the backscattered electrons (i.e. the spatial resolution) equals 590 nm. Semi-quantitative El/Ca_{cc} profiles were calculated by averaging the El/Ca_{cc} intensities parallel to the banding direction and applying a constant calibration factor obtained from LA-ICP-MS measurements on the same specimen, similar to the procedure of Eggins et al. (2004). We did not use the depth-resolved laser ablation-profiles for this purpose, but used the average value from the profiles for correlation to the EPMA-derived intensities.

2.3 Results

2.3.1 Foraminiferal calcite element ratios and partitioning coefficients as a function of salinity

Per treatment, from lowest to highest salinity, average Na/Ca_{cc} of the newly formed calcite varied between 9.3 and 10.8 mmol/mol for *A. lessonii* and between 4.7 and 6.4 mmol/mol (highest salinity) for *A. tepida* (Fig. 1), with a corresponding partition coefficient (note that partition coefficients are 'apparent', not taking into account speciation/activity of Na) ranging from 1.90×10^{-4} to 2.20×10^{-4} and from 0.97×10^{-4} to 1.30×10^{-4} for *Amphistegina* and *Ammonia*, respectively (Table 3). For both species, sets of single-specimen Na/Ca_{cc} show slightly skewed distributions towards higher Na/Ca_{cc} for all salinities (Kolmogorov-Smirnov test, at the 95% confidence level). Combining all specimens (based on the average of single-spot measurements per specimen), Na/Ca_{cc} shows a positive linear relationship with salinity for both *A. lessonii* and *A. tepida* ($Na/Ca_{cc} = 0.077 \pm 0.017 \times S + 7.13 \pm 0.60$, $F_{1,186} = 20.9$, $p < 0.001$ for *A. lessonii* and $Na/Ca_{cc} = 0.064 \pm 0.013 \times S + 3.29 \pm 0.44$, $F_{1,172} = 25.9$, $p < 0.001$ for *A. tepida*; Fig. 1). The observed average relative standard deviation between specimens in Na/Ca_{cc} at each of the 5 salinities is 15% for *A. lessonii* and 20% for *A. tepida*. The variance in Na/Ca_{cc} between individual specimens explained by salinity is $\eta^2 = 0.08$ for *A. lessonii* and $\eta^2 = 0.14$ for *A. tepida*.

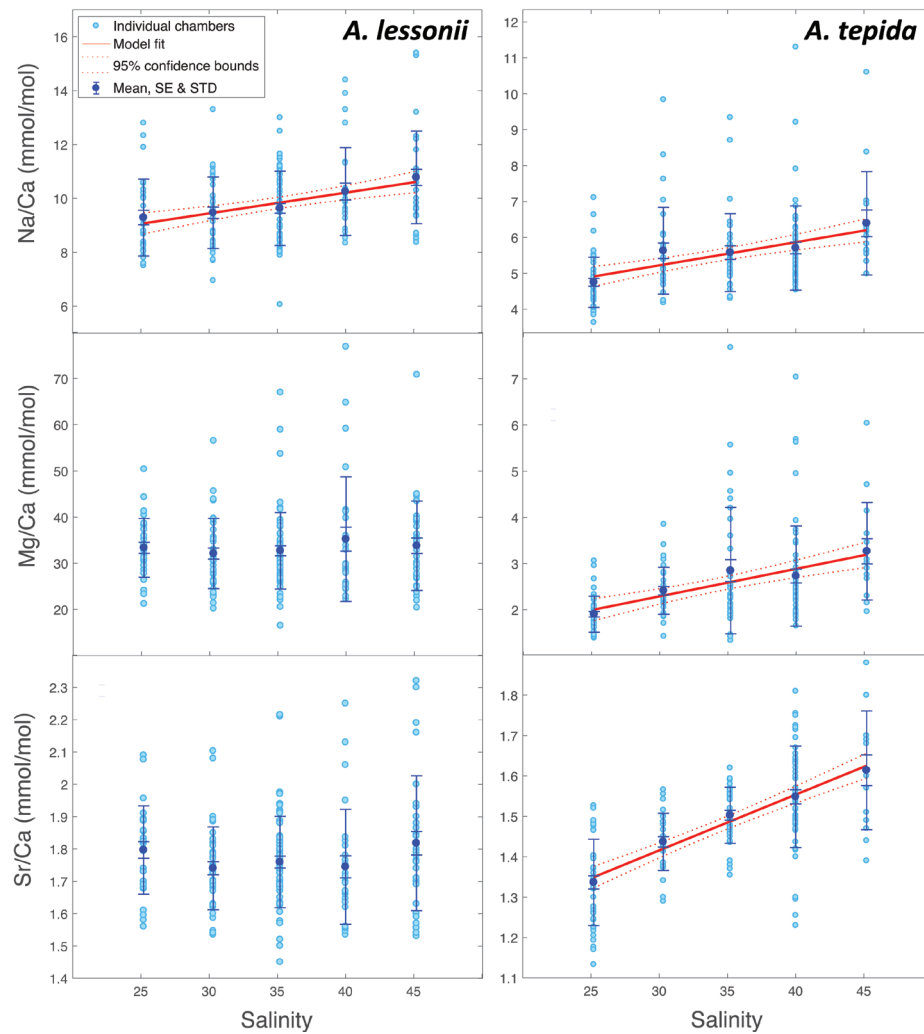


Figure 1. Foraminiferal Na/Ca_{cc}, Mg/Ca_{cc} and Sr/Ca_{cc} versus salinity. Light blue dots represent the average per specimen ($n = 359$ for *A. lessonii*, $n = 339$ for *A. tepida*, with 2-3 measured chambers per individual), dark blue dots indicate the mean, with inner error bars indicating the standard error and outer error bars the standard deviation for each treatment. The linear regression model (red line) is based on the individuals' mean, with the 95% confidence interval of the regression in dashed lines.

Specimen's average Mg/Ca_{cc} and Sr/Ca_{cc} correlate positively with salinity in *A. tepida* ($\text{Mg/Ca}_{\text{cc}} = 0.060 \pm 0.011 \times S + 0.51 \pm 0.38$, $F_{1,172} = 29.9$, $p < 0.001$ and $\text{Sr/Ca}_{\text{cc}} = 0.014 \pm 12 \times 10^{-4} \times S + 1.00 \pm 0.04$, $F_{1,337} = 254$, $p < 0.001$), whereas neither ratio correlates with salinity in *A. lessonii*. Average relative standard deviations for the 5 salinity conditions per element are 27% for Mg/Ca_{cc} and 9% for Sr/Ca_{cc} in *A. lessonii* and 32% in Mg/Ca_{cc}

and 7% for Sr/Ca_{cc} for *A. tepida*. In *A. lessonii*, the proportion of variance in Sr/Ca_{cc} explained by salinity is $\eta^2 = 0.04$ ($p < 0.01$) (Mg/Ca_{cc} not significant) and for *A. tepida*, the proportion of variance in Sr/Ca_{cc} explained by salinity is $\eta^2 = 0.44$ and in Mg/Ca_{cc} $\eta^2 = 0.19$ ($p < 0.001$).

Single-spot analyses on *Ammonia tepida* show that Na/Ca_{cc} and Mg/Ca_{cc} are significantly correlated within the salinity treatments, except for condition S=30 (Fig. 2). For the individual salinity treatments, single-spot Sr/Ca_{cc} and Mg/Ca_{cc}, as well as Na/Ca_{cc} and Sr/Ca_{cc} are not correlated significantly with each other, except for S=25. Between salinity treatments, distributions in this species shift towards higher Na/Ca_{cc}, Sr/Ca_{cc} and Mg/Ca_{cc} values with increasing salinity, although for the range between 30-40 Na/Ca_{cc} distributions remain rather similar (Fig. 2). For *Amphistegina lessonii*, distributions of Sr/Ca_{cc} and Mg/Ca_{cc} ratios overlap largely between salinities, and only Na/Ca_{cc} distributions shift towards higher values (Fig. 2). Within each salinity condition however, single-spot Na/Ca_{cc}, Mg/Ca_{cc} and Sr/Ca_{cc} in this species are positively correlated amongst each other, whereby the Na/Ca_{cc} intercept of these relationships increases with increasing salinity (Fig. 2 and Appendix C).

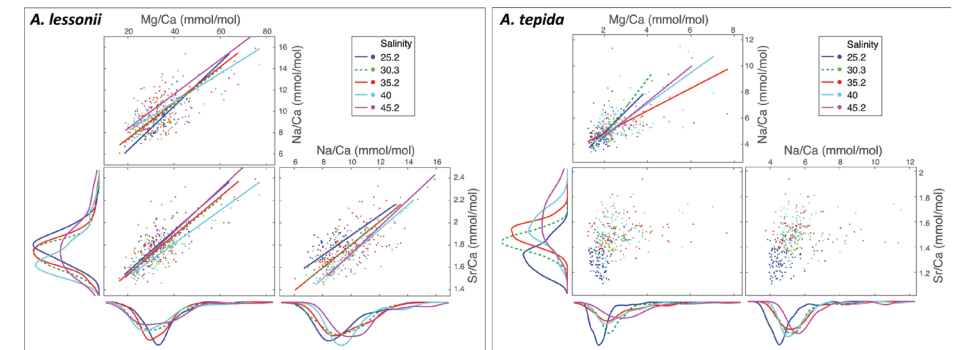


Figure 2. Individual chamber LA-ICP-MS analyses showing correlations between foraminiferal Mg/Ca_{cc}, Sr/Ca_{cc} and Na/Ca_{cc} for *A. tepida* (left) and *A. lessonii* (right) per salinity condition. Significant orthogonal linear regressions are indicated with a line, colour coded for salinity (see legend). Correlation coefficients, slope and intercepts of these regressions can be found in Appendix C. In short, within salinity conditions, element ratios are strongly correlated with each other in *A. lessonii*, whereas in *A. tepida*, element ratios do not (strongly) correlate with each other. When combining all single-spot data in *A. tepida*, element ratios correlate amongst each other because the incorporation of all three elements increases with salinity, shifting the distributions to higher values. In *A. lessonii*, only the Na/Ca_{cc} distributions shift towards higher values with increasing salinity, whereas Mg/Ca_{cc} and Sr/Ca_{cc} distributions are relatively similar between salinity conditions.

Table 3. Average $\text{El}/\text{Ca}_{\text{cc}}$ ratios of the foraminiferal calcite (based on average of average specimens value per salinity (Sal) condition (S25-S45) $\pm$ standard error and corresponding apparent partitioning coefficients, defined as $D_{\text{El}} = (\text{El}/\text{Ca}_{\text{cc}})/(\text{El}/\text{Ca}_{\text{seawater}})$ for *A. lessonii* (*A.l.*) and *A. tepida* (*A.t.*). 'n/spots' stands for number of specimens and average number of spots per specimen.

Sal	n/spots	$\text{Na}/\text{Ca}_{\text{cc}}$ mmol/mol	D_{Na}	$\text{Mg}/\text{Ca}_{\text{cc}}$ mmol/mol	D_{Mg}	$\text{Sr}/\text{Ca}_{\text{cc}}$ mmol/mol	D_{Sr}
<i>A.l.</i>							
S25	65/2.6	9.29 $\pm$ 0.27	1.90 $\times 10^{-4}$	33.35 $\pm$ 1.20	5.94 $\times 10^{-3}$	1.80 $\pm$ 0.026	0.199
S30	74/1.9	9.47 $\pm$ 0.21	1.90 $\times 10^{-4}$	32.10 $\pm$ 1.20	5.64 $\times 10^{-3}$	1.74 $\pm$ 0.020	0.189
S35	103/1.9	9.63 $\pm$ 0.18	1.98 $\times 10^{-4}$	32.71 $\pm$ 1.07	5.94 $\times 10^{-3}$	1.76 $\pm$ 0.018	0.191
S40	50/2	10.25 $\pm$ 0.31	2.11 $\times 10^{-4}$	35.22 $\pm$ 2.60	6.27 $\times 10^{-3}$	1.74 $\pm$ 0.034	0.184
S45	44/1.4	10.78 $\pm$ 0.30	2.20 $\times 10^{-4}$	33.80 $\pm$ 1.68	5.90 $\times 10^{-3}$	1.82 $\pm$ 0.036	0.189
<i>A.t.</i>							
S25	109/2.5	4.75 $\pm$ 0.11	0.97 $\times 10^{-4}$	1.90 $\pm$ 0.06	3.40 $\times 10^{-4}$	1.34 $\pm$ 0.016	0.148
S30	58/1.8	5.63 $\pm$ 0.22	1.13 $\times 10^{-4}$	2.41 $\pm$ 0.09	4.24 $\times 10^{-4}$	1.44 $\pm$ 0.013	0.156
S35	59/1.8	5.58 $\pm$ 0.19	1.15 $\times 10^{-4}$	2.85 $\pm$ 0.24	5.17 $\times 10^{-4}$	1.50 $\pm$ 0.012	0.163
S40	93/1.8	5.70 $\pm$ 0.16	1.17 $\times 10^{-4}$	2.73 $\pm$ 0.15	4.86 $\times 10^{-4}$	1.55 $\pm$ 0.017	0.164
S45	201.3	6.39 $\pm$ 0.37	1.31 $\times 10^{-4}$	3.27 $\pm$ 0.27	5.70 $\times 10^{-4}$	1.61 $\pm$ 0.038	0.168

2.3.2 Size and chamber effect on $\text{Na}/\text{Ca}_{\text{cc}}$ and inter-specimen variance

Specimens of *A. lessonii* produced most new chambers at salinities of 25, 30 and 35, closest to the salinity in their “natural” habitat (Burgers Zoo aquarium, salinity (33.9–34.3; Ernst et al., 2011). Size averages are not significantly different between these salinity treatments, based on a Kruskal-Wallis test, whereas specimens grown at salinities 40 and 45 were significantly smaller than those from lower salinities, reflecting lower chamber addition rates over the course of the culturing experiment at higher salinity (Fig. 3). Combining all specimens, $\text{Na}/\text{Ca}_{\text{cc}}$ is not significantly related to size in *A. lessonii*. Specimens of *A. tepida* produced less chambers at salinity 45, possibly because such a high salinity is probably close to its tolerance levels (Murray, 2014), even though this species is adapted to relatively large salinity shifts in their tidal flat habitat. Specimens in the lower salinity groups (25, 30, 35) grew larger compared to specimens grown at two the highest salinity groups (Fig. 3). Combining all specimens, $\text{Na}/\text{Ca}_{\text{cc}}$ is significantly related to size in *A. tepida*, yet with a small slope (-0.003) and just within the 95% confidence interval ($p=0.04$).

Within each salinity tested, single-chambered $\text{Na}/\text{Ca}_{\text{cc}}$ is slightly positively related to size for the specimens of *A. lessonii* cultured at salinities 25 (slope = 0.008, $R^2 = 0.32$, $p < 0.01$), 30 (slope = 0.002, $R^2 = 0.11$, $p < 0.05$ and 35 (slope = 0.005, $R^2 = 0.18$, $p < 0.001$). For the same species, $\text{Mg}/\text{Ca}_{\text{cc}}$ is positively correlated to size at salinities 25, 30 and 35, with a similar slope of 0.03 ($p < 0.05$). $\text{Sr}/\text{Ca}_{\text{cc}}$ also shows a positive relationship

to size within salinities 25, 30 and 35 with slopes of 0.0007, 0.0003, 0.0005 ($p < 0.001$) respectively. For *A. tepida*, there is only a slight negative correlation between size and $\text{Sr}/\text{Ca}_{\text{cc}}$ for specimens cultured at salinity 25 (slope = 9.9×10^{-4} , $p < 0.001$) and no significant correlation for the other conditions, or between size and $\text{Na}/\text{Ca}_{\text{cc}}$ and $\text{Mg}/\text{Ca}_{\text{cc}}$ in any of the salinities.

At the lowest salinity, $\text{Na}/\text{Ca}_{\text{cc}}$ in the F-chamber (newest chamber) show slight (0.9 mmol/mol Na/Ca higher median) but significant higher values than the F-2 chambers for *A. lessonii* (multicompare test based on Kruskal-Wallis test, $p < 0.05$). For specimens of *A. lessonii* cultured at other salinities and for *A. tepida* at any of the salinities tested, there no significant correlations between $\text{Na}/\text{Ca}_{\text{cc}}$ and chamber position were observed (note that only chamber positions F to F-2 were taken into account, as for the lower chamber position sample numbers were insufficient). Furthermore, chamber position shows no significant effect on $\text{Mg}/\text{Ca}_{\text{cc}}$ and $\text{Sr}/\text{Ca}_{\text{cc}}$.

To further investigate the variance between and within individuals, a multiway ANOVA was performed to investigate the effect on $\text{Na}/\text{Ca}_{\text{cc}}$ per salinity condition. Inter-individual variance is significant and larger than the variance between chamber groups and intra-individual variance in all salinity groups, with the between individual variability accounting for $\eta^2 = 0.75 \pm 0.11 / 0.84 \pm 0.03$ of the variance ($p < 0.001$) for *A. lessonii* and *A. tepida*, respectively. The variance due to chamber position is not significant and the remaining intra-individual variance accounts for $\eta^2 = 0.09 \pm 0.05 / 0.08 \pm 0.05$ for *A. lessonii* and *A. tepida*, respectively.

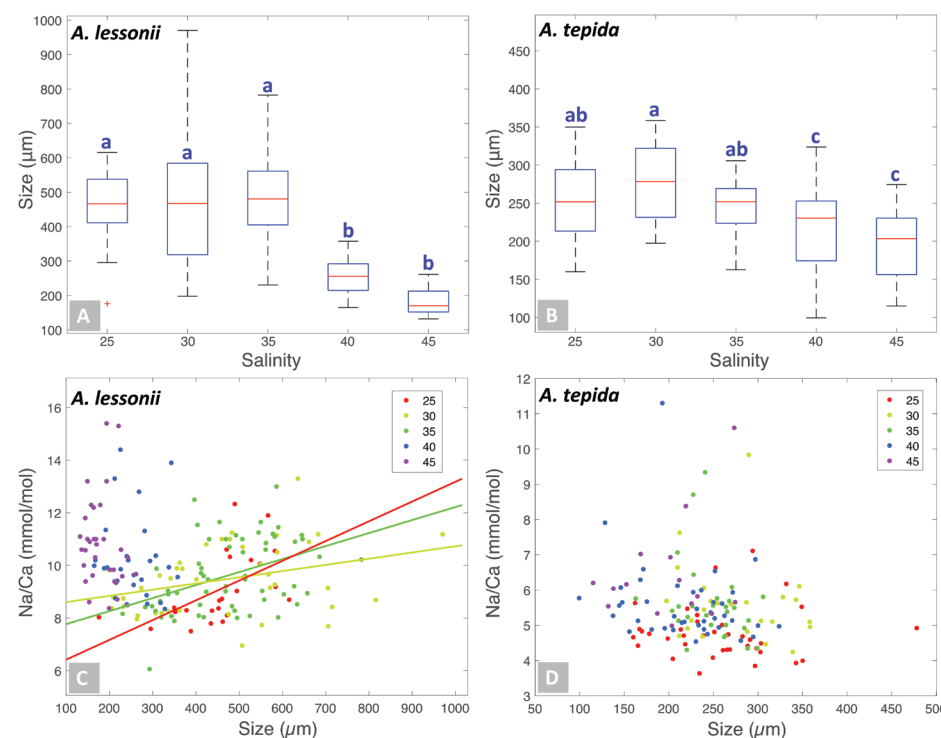


Figure 3. Boxplots (Panel A and B) showing the size distributions (median, 1st and 3rd quartiles, minimum and maximum values) for each salinity condition, $n = 24, 40, 60, 27, 33$ for *A. lessonii* and $n = 38, 24, 28, 41, 15$ for *A. tepida*. Letters (a, b, c) indicate significant different population means, based on ANOVA ($p < 0.001$). Panel C and D show the Na/Ca values against size measurements per individual, colour coded per salinity condition (see legend), for *A. lessonii* and *A. tepida*. Significant linear regression lines are plotted for *A. lessonii*.

2.3.3 Elemental distributions in the chamber wall

EPMA maps of cross-sectioned chamber walls of *A. lessonii* show, within the resolution limits of the technique, that bands of elevated Na/Ca_{cc} intensities overlap with zones of elevated Mg/Ca_{cc} (Fig. 4 and Appendix D). Mg bands show higher amplitudes than Na bands, but clearly coincide spatially. Comparing EPMA maps with the backscatter SEM image of the exposed sections shows that the bands with the highest Na/Ca_{cc} and Mg/Ca_{cc} occur in the proximity of the organic linings, which are clearly visible in the backscatter SEM image (Fig. 4), with a number of high Na- and Mg-rich bands with slightly lower maximum intensities occurring towards the outer chamber surface coinciding with subsequent organic linings. For *A. tepida*, one band of elevated Mg/Ca_{cc} band is visible coinciding with the POS with no clear Na/Ca_{cc} banding being detected.

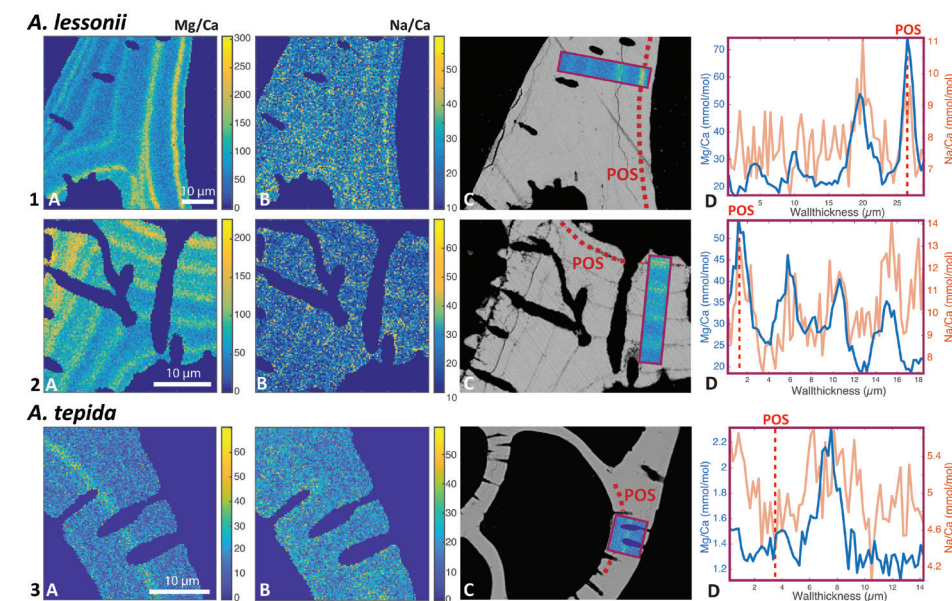


Figure 4. Foraminiferal Mg/Ca_{cc} (A panels; left) and Na/Ca_{cc} (B panels) intensity ratio maps, obtained with EPMA, for two specimens of *A. lessonii* grown at a salinity of 30 (row 1) and 25 (row 2) and one specimen of *A. tepida* (row 3). D panels (right) show profiles for Mg/Ca (blue) and Na/Ca (red), based on averaged EPMA ratios scaled to LA-ICP-MS measurements of the same specimen, of an averaged lateral profile area through the chamber wall perpendicular to the lamella separated by organic linings (purple rectangles C). The transect area is indicated with a purple rectangle, on top of a backscatter SEM image (C), showing that the high El/Ca bands overlap with the primary organic sheet (POS, marked with dashed red line) and subsequent organic linings. See Appendix D for the results for three more specimens.

2.4 Discussion

2.4.1 The effect of salinity and DIC on Na/Ca_{cc}, Mg/Ca_{cc} and Sr/Ca_{cc}

The single-specimen Na/Ca_{cc} data of the cultured *A. lessonii* and *A. tepida* both correlate positively with salinity (Table 3, Fig. 1). This is in line with previous calibrations (for *Ammonia tepida*, Wit et al., 2013; for cultured *Globigerinoides ruber*, Allen et al., 2016; for field-collected *G. ruber* and *G. sacculifer*, Mezger et al., 2016). However, our Na/Ca_{cc}-salinity calibration for *A. tepida* is somewhat less sensitive than that observed earlier for the same species (Wit et al., 2013). An offset in Na/Ca_{cc} values between calibrations for a single species has been reported previously for the planktonic *G. ruber* and *G. sacculifer* (e.g. Mezger et al., 2016; Allen et al., 2016). Such an apparent discrepancy between studies may be caused by differences between cultures or in situ conditions in one of the conditions not focussed on (e.g. carbon chemistry, light intensity). Alternatively, subtle analytical differences (e.g. differences in cleaning procedures), statistical reasons (for

example differences in the number of analyses or sample size) or the effect of genotypic variability on element incorporation (Sadekov et al., 2016) may also play a role. Although the calibration presented here consists of many more data points compared to those in Wit et al. (2013), we do not want to dismiss the latter as several parameters (like cleaning procedures or the source of the seawater used for the culture media) inherently vary (marginally) between studies. As such the difference observed between studies merely illustrates the potential range for this species.

Contrasts in sensitivities such as observed for $\text{Na}/\text{Ca}_{\text{cc}}$ between calibrations also apply to $\text{Mg}/\text{Ca}_{\text{cc}}$ and $\text{Sr}/\text{Ca}_{\text{cc}}$, both of which here show an increase with salinity in *A. tepida* but not in *A. lessonii* (Fig. 1). Previous culturing experiments with *Ammonia tepida*, however, showed a smaller sensitivity of $\text{Mg}/\text{Ca}_{\text{cc}}$ to salinity (0.029-0.0044 mmol/mol change per salinity unit; Dissard et al., 2010b) than that reported here (0.06). Still, all these sensitivities are considerably lower than that reported in Kısakürek et al. (2008) for the planktonic *G. ruber* (0.23 when $\text{Mg}/\text{Ca}_{\text{cc}}$ is assumed to increase linearly with salinity), but in the same range as that reported by Nürnberg et al. (1996) for *G. sacculifer* (0.05). The sensitivity of $\text{Sr}/\text{Ca}_{\text{cc}}$ to salinity in *A. tepida* (0.014; Table 3) is comparable to that for *O. universa* (0.008; Lea et al., 2008), *G. ruber* (0.02; Kısakürek et al., 2008) and similar to the significant effect of salinity on Sr incorporation in the same species (0.01-0.02, depending on temperature) found by Dissard et al. (2010b).

Seawater carbonate chemistry is an additional factor potentially affecting trace metal uptake (e.g. Keul et al., 2017; Lea et al., 1999; Russell et al., 2004). Since salinity and dissolved inorganic carbon concentration in the culture media co-varied in our experiments similar to the natural environment (Table 1), $\text{Na}/\text{Ca}_{\text{cc}}$ in our cultured specimens also correlates positively to seawater [DIC]. However, sodium incorporation has been shown to be independent from changes in carbonate chemistry in cultured *Amphistegina gibbosa* and several other benthic hyaline and porcelaneous species (Van Dijk et al., 2017b). Additionally, Allen et al., (2016) also found no significant effect of carbonate chemistry (i.e. varying $[\text{CO}_3^{2-}]$) on Na incorporation in cultured *G. ruber*, suggesting that the variability in $\text{Na}/\text{Ca}_{\text{cc}}$ observed here in *A. lessonii* can be attributed to changes in salinity rather than [DIC]. However, future studies should disentangle the impacts of DIC and salinity on Na/Ca, in order to increase proxy confidence in areas where Na/Ca and DIC relationships differ from the global average. Previous studies showed that $\text{Sr}/\text{Ca}_{\text{cc}}$ correlates positively to [DIC] in *A. tepida* (Keul et al., 2017), which may account for part of the correlation between $\text{Sr}/\text{Ca}_{\text{cc}}$ and salinity reported here for this species. The published sensitivity of $\text{Sr}/\text{Ca}_{\text{cc}}$ to [DIC] is approximately 2×10^{-5} mmol/mol change in $\text{Sr}/\text{Ca}_{\text{cc}}$ for every 1 $\mu\text{mol}/\text{kg}$ change in [DIC], likely representing the maximum

potential effect of DIC on Sr partitioning given that others found no significant effect (Dissard et al., 2010a). For a change in $\sim 850 \mu\text{mol}/\text{kg}$ (Table 1), this would amount to an increase in $\text{Sr}/\text{Ca}_{\text{cc}}$ of 0.019 mmol/mol (Keul et al., 2017) over the salinity range studied here, thereby accounting for approximately 7% of the total observed change in $\text{Sr}/\text{Ca}_{\text{cc}}$ (Table 3). Inorganic carbon chemistry is known to affect growth rates and shell weights in benthic foraminifera (Dissard et al., 2010a; Keul et al., 2013a), which in turn, may affect incorporation of Sr and Mg, hence providing a mechanistic link between inorganic carbon chemistry and element partitioning.

El/Ca ratios of specimens of both species grown within each salinity condition are characterized by a relatively large variability. In the overall data set, salinity only explains around 8% of the variation in Na incorporation for *A. lessonii* and 14%, 19% and 44% of Na, Mg and Sr incorporation in *A. tepida*. Nevertheless, for *A. lessonii*, the Na/Ca mean values (which translates to the values obtained from traditional solution-ICP-MS) fit the regression model relatively well (Fig. 1). However, given the low sensitivity, many specimens are required to reduce the uncertainty (Appendix E). This is reflected by the relatively wide prediction bounds for the Na/Ca-salinity regressions, indicating an uncertainty associated with a single $\text{Na}/\text{Ca}_{\text{cc}}$ measurement. The relatively large inter-specimen variability in element/ Ca_{cc} ratios has been reported and discussed before (e.g. De Nooijer et al., 2014a; Sadekov et al., 2008), but the cause for this variability remains to be identified.

2.4.2 Inter-specimen, inter-species and intra-shell El/ Ca_{cc} variability

Single-chamber measurements show that $\text{Na}/\text{Ca}_{\text{cc}}$ for both species varies between chambers (i.e. specimens) with a RSD (Relative Standard Deviation) of 15%-20%, despite identical culture conditions (Fig. 1). Since the analytical error on $\text{Na}/\text{Ca}_{\text{cc}}$ accounts for approximately 2% (Table 2), a large portion of the observed variability between specimens must be due to ontogeny and/or inter-specimen differences in biomineralization controls (De Nooijer et al., 2014a).

Foraminiferal shell size at salinities 40 and 45 are significantly smaller than those cultured at lower salinities. When combining data from all salinities, however, there is no (*A. lessonii*) or only a very small (*A. tepida*) negative correlation between $\text{Na}/\text{Ca}_{\text{cc}}$ and shell size, as opposed to a more substantial negative correlation as observed by Wit et al. (2013). In fact, there appears to be a growth optimum around salinity 30-35, whereas growth at higher salinities might be hampered (Fig. 3). This may indicate that the earlier observed negative correlation between size and Na/Ca was the result of indirect co-variation with salinity rather than a causal relationship resulting in lower Na/Ca values

in smaller specimens. This is corroborated by our observation that, for individuals grown at a similar salinity, the relationship between $\text{Na}/\text{Ca}_{\text{cc}}$ and size is either slightly positive or absent. Hence, size unlikely affects the observed inter-specimen variability in $\text{Na}/\text{Ca}_{\text{cc}}$, which is supported by the absence of a correlation between chamber position (and hence ontogenetic stage) and $\text{Na}/\text{Ca}_{\text{cc}}$. This implies that measuring specimens of different size fractions or measuring different or multiple chambers should not significantly affect the application of the $\text{Na}/\text{Ca}_{\text{cc}}$ salinity proxy. However, sufficient specimens ($n > 30$, for an error margin $< 5\%$ at the 95% confidence level; Sadekov et al., 2008; De Nooijer et al., 2014a) are required for measurements. As most variability is between individuals rather than between chambers (section 3.3), analyzing more chambers of the same specimen would increase the accuracy of the measurement, but not improve the precision of the salinity estimate, given the large inter-specimen variability. Without a major effect of ontogeny, physiological processes at the organismal level are more likely to cause observed large inter-specimen variability in $\text{Na}/\text{Ca}_{\text{cc}}$, however these processes remain poorly understood.

In *A. lessonii*, single-spot $\text{Na}/\text{Ca}_{\text{cc}}$, $\text{Sr}/\text{Ca}_{\text{cc}}$ and $\text{Mg}/\text{Ca}_{\text{cc}}$ are correlated amongst each other within each salinity condition (Fig. 2). Correlation coefficients between the three element ratios are similar for the different salinities, with superimposed an increase in the $\text{Na}/\text{Ca}_{\text{cc}}$ relative to that of $\text{Mg}/\text{Ca}_{\text{cc}}$ and $\text{Sr}/\text{Ca}_{\text{cc}}$ with increasing salinity (Appendix C). In contrast, single-spot $\text{Sr}/\text{Ca}_{\text{cc}}$ and $\text{Mg}/\text{Ca}_{\text{cc}}$ in *A. tepida* are not correlated, whereas incorporation of all these elements increases significantly with salinity. Within salinities $\text{Mg}/\text{Ca}_{\text{cc}}$ and $\text{Na}/\text{Ca}_{\text{cc}}$ are significantly correlated in 4 out of the 5 salinities, but with much lower correlation coefficients compared to *A. lessonii* (Fig. 2 and Appendix C). However, between the different salinities these elements are correlated in *A. tepida*, implying that for *A. tepida* salinity is one of the actual parameters controlling $\text{Na}/\text{Ca}_{\text{cc}}$, $\text{Mg}/\text{Ca}_{\text{cc}}$ and $\text{Sr}/\text{Ca}_{\text{cc}}$ element uptake.

Within conditions, the correlations between both $\text{Sr}/\text{Ca}_{\text{cc}}$ and $\text{Na}/\text{Ca}_{\text{cc}}$ with $\text{Mg}/\text{Ca}_{\text{cc}}$ in *A. lessonii* differ from the correlation of $\text{Sr}/\text{Ca}_{\text{cc}}$ with $\text{Mg}/\text{Ca}_{\text{cc}}$ (correlation absent) and $\text{Na}/\text{Ca}_{\text{cc}}$ with $\text{Sr}/\text{Ca}_{\text{cc}}$ (weaker correlation) for *A. tepida*. The differences between the correlations likely reflects differences in their calcification pathway (e.g. transport of ions to the site of calcification) and/or might be explained by differences in lattice strain due to the higher Mg-content in *A. lessonii*, whereas this effect is expected to be less prominent in low-Mg species such as *A. tepida* (Evans et al., 2015). Differences in the calcification pathway may also explain why $\text{Sr}/\text{Ca}_{\text{cc}}$ and $\text{Mg}/\text{Ca}_{\text{cc}}$ are correlated to salinity in *A. tepida*, but not in *A. lessonii* (4.1).

In both species, Mg is found to be elevated in bands located close to the primary organic sheet and to other organic layers (Fig. 4), present in rotaliid species due to their lamellar calcification mode (Reiss, 1957, 1960). This is similar to reports of within-chamber wall banding in many elements in other species (Branson et al., 2016; Eggins et al., 2004; Fehrenbacher et al., 2017; Hathorne et al., 2009; Kunioka et al., 2006; Paris et al., 2014; Sadekov et al., 2005; Spero et al., 2015; Steinhardt et al., 2015). In planktonic species element banding has been related to diurnal light-dark cycles rather than the addition of a new lamella with chamber addition (Spero et al., 2015; Fehrenbacher et al., 2017). Whether in the species studied here, chamber addition (and hence element banding) is related to day night cycles remains to be investigated. As in other studies, the Na- and Mg- bands are spatially correlated (Fig. 4). For *Ammonia tepida*, the banding in both elements is less pronounced than for *Amphistegina lessonii*, which is likely related to the (much) lower average $\text{Mg}/\text{Ca}_{\text{cc}}$ and $\text{Na}/\text{Ca}_{\text{cc}}$ ratios in the former species. Alternatively, as the observations are close to the spatial resolution of the method, the observed pattern could also be due to the band's width being smaller in *A. tepida* compared to *A. lessonii*.

2.4.3 Biomineralization controls on element uptake

How elements are transported to the site of calcification and what is the role of seawater-vacuolization, -leakage, trans-membrane-transport of ions, pH regulation and precipitation rate and how this differs between species and specimens, remains to be discovered. The overall element composition of the calcite precipitated by *A. lessonii* suggests that the calcification process of this species may more closely resemble inorganic calcite precipitation from seawater, compared to that in *Ammonia tepida* and other low-Mg calcite precipitating species. As a result, more elements (like Mg) are incorporated and crystal lattice strain in intermediate-Mg calcite species is elevated, which may promote incorporation of other elements through stress compensation (Mewes et al., 2015; Mucci and Morse, 1983). This would explain the observed inter-element correlations within salinities. Another difference between the species studied here may be caused by differences in CaCO_3 phase shifts during calcite precipitation (e.g. Bots et al., 2012; De Yoreo et al., 2015). A metastable vaterite pre-cursor phase recently found in two planktonic species may explain the low Mg incorporation relative to inorganic calcite (Jacob et al., 2017). The higher Mg contents of *A. lessonii* could be related to the (partial) absence of a vaterite-calcite transformation in this species. An Amorphous Calcium Carbonate (ACC) pre-cursor phase has been observed in other marine biomineralising organisms (e.g. Giuffrè et al., 2015; Weiner et al., 2003) and often been hypothesised to play a role in foraminiferal calcification (De Nooijer et al., 2014b; Erez, 2003), although it has not yet been directly detected. A higher Mg concentration at the site of calcification could hypothetically result in a phase shift from amorphous

calcium carbonate (ACC) directly into calcite, whereby Mg is stabilizing the ACC, as described by Littlewood et al. (2017). In inorganic calcite, the absence of a vaterite precursor phase also enhances the incorporation of other metals incompatible to calcite, such as Sr (Littlewood et al., 2017) and a similar process could hypothetically contribute to inter-species differences in element partitioning similar to that observed here. Although the strong fractionation against Mg in *A. tepida* could reflect double fractionation through a vaterite-calcite transformation (Jacob et al., 2017) the low-Mg content might as well reflect a more enclosed site of calcification, whereby ions are mainly transported trans-membrane (Nehrke et al., 2013; De Nooijer et al., 2014b). However, the experiments here do not allow distinguishing between these (and other) potential mechanisms. Trans-membrane transport (TMT) of Ca^{2+} and concomitant leakage of Mg^{2+} and Sr^{2+} might be more sensitive to differences in ionic strength and element concentrations, hence possibly explaining the salinity effect on the incorporation of these elements in *A. tepida* whereas it does not in *A. lessonii*, assuming that TMT relatively contributes more to the supply of ions to the site of calcification in this species compared to *A. lessonii*, which might be relatively more dependent on seawater vacuolisation. However, since there are many, both biotic and abiotic, mechanisms that can (simultaneously) influence (coupled) element partitioning, it is challenging to resolve the exact mechanism responsible for inter-specimen and inter-species differences in El/Ca .

The spatial correlation between the intra-shell distributions of Mg and Na, associated to the organic linings, suggests a coupled control on these elements during the calcification process, which is in line with the observed inter-specimen correlations. This suggests that incorporation of these cations is influenced by similar biomineralization mechanisms, related to seawater vacuolization (Bentov and Erez, 2006; Erez, 2003), trans-membrane transport of elements (Nehrke et al., 2013), lattice-strain effect (Evans et al., 2015) and/or metastable precursor phases (Jacob et al., 2017). The relative contributions of these mechanisms might differ between species, resulting in the observed differences in element incorporation and different inter-element correlations between species. Differences in the efficiency of such processes between specimens might cause the observed inter-specimen variability, whereas changes in these processes during the calcification time could be responsible for the observed correlation between elements within the chamber wall.

2.5 Conclusions

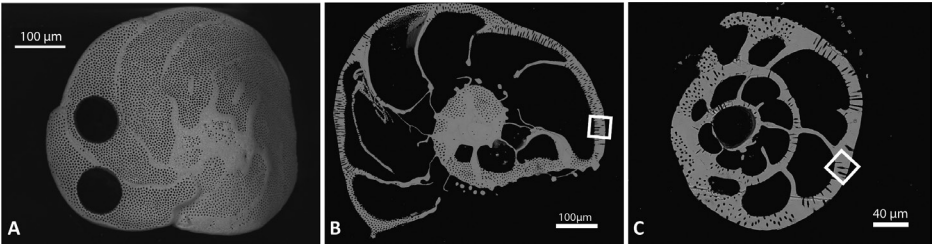
By extending existing calibrations of the $\text{Na}/\text{Ca}_{\text{cc}}$ -salinity proxy to the intermediate-Mg calcite precipitating benthic foraminifer *Amphistegina lessonii*, we show that the $\text{Na}/\text{Ca}_{\text{cc}}$ increase as a function of salinity is similar to that in previously studied species.

The absolute $\text{Na}/\text{Ca}_{\text{cc}}$ for *A. lessonii* is, however, higher than that in *Ammonia tepida*. In *A. tepida*, $\text{Mg}/\text{Ca}_{\text{cc}}$ and $\text{Sr}/\text{Ca}_{\text{cc}}$ are positively correlated to salinity, whereas they are not impacted by salinity in *A. lessonii*. Within each salinity, single chamber- $\text{Na}/\text{Ca}_{\text{cc}}$ and $\text{Mg}/\text{Ca}_{\text{cc}}$ are positively correlated in *A. tepida*, whereas single chamber- $\text{Sr}/\text{Ca}_{\text{cc}}$ is not correlated to either $\text{Mg}/\text{Ca}_{\text{cc}}$ or Na in this species. For *A. lessonii*, all $\text{Sr}/\text{Ca}_{\text{cc}}$, $\text{Mg}/\text{Ca}_{\text{cc}}$ and $\text{Na}/\text{Ca}_{\text{cc}}$ combinations are positively correlated at the single chamber level. Electron Microprobe Analysis mapping of Na and Mg within chamber walls of cultured specimens shows that in *A. lessonii*, $\text{Na}/\text{Ca}_{\text{cc}}$ and $\text{Mg}/\text{Ca}_{\text{cc}}$ occur in elevated bands in close proximity to the primary organic lining. For specimens of *A. tepida*, Mg-banding appears similar to that in *A. lessonii*, whereas Na-banding is less prominent in this species. These results suggest that biomineralization controls on incorporated elements differ between species.

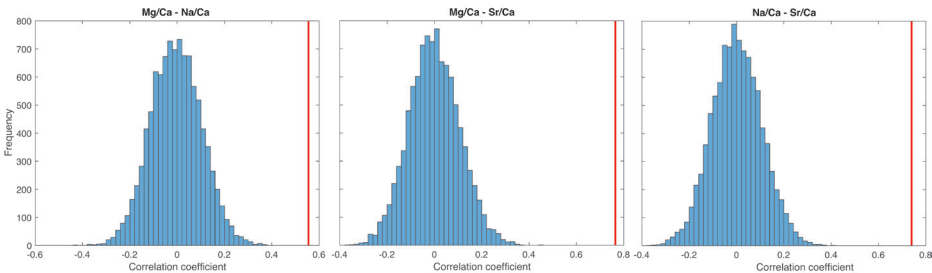
Acknowledgements

We would like to thank Wim Boer for assistance with LA-ICP-MS measurements, Patrick Laan for seawater measurements and Karel Bakker for DIC measurements. We kindly thank Max Janse (Burgers' Zoo, Arnhem) for providing stock specimens of *A. lessonii* and Kirsten Kooijmans (NIOZ) for providing cultures of *Dunaliella salina*. Sergei Matveev is thanked for assistance with the Electron Microprobe analysis and Leonard Bik for assistance with polishing the samples. This work was carried out under the program of the Netherlands Earth System Science Centre (NESSC), financially supported by the Ministry of Education, Culture and Science (OCW) (Grantnr. 024.002.001) and Darwin Centre for Biogeosciences (program 3020).

Appendix



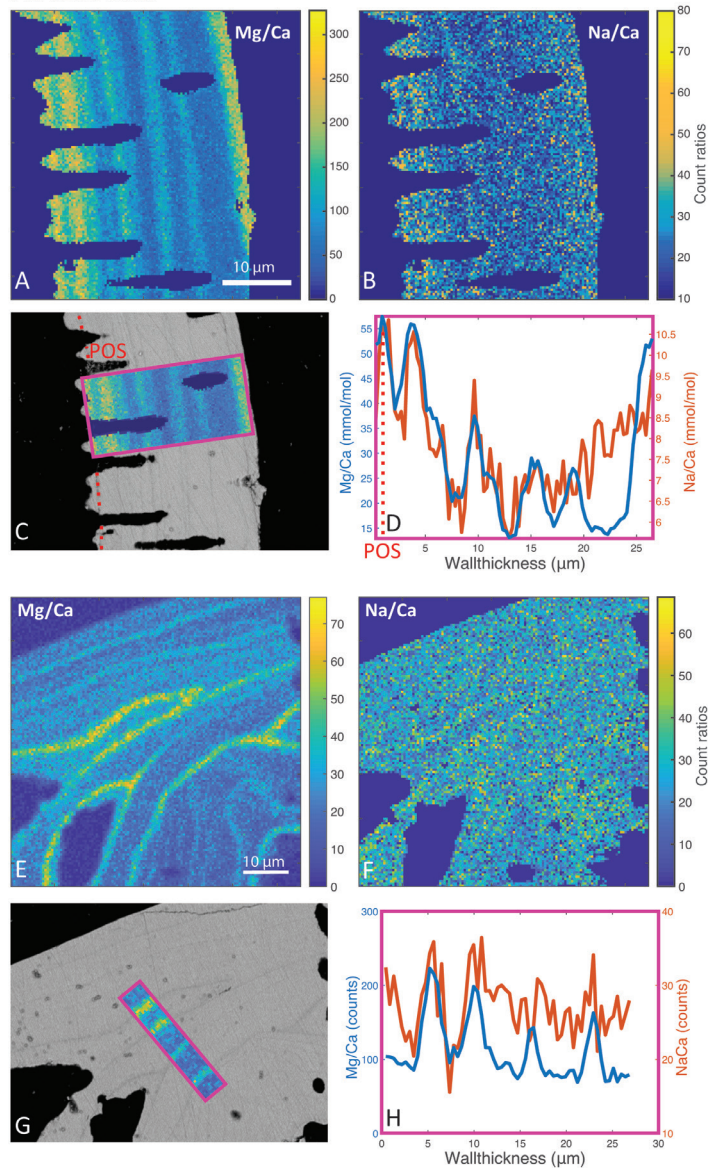
Appendix A. SEM image of a specimen of *A. lessonii* showing LA-ICP-MS measurement spots (panel A) and SEM images of specimens of *A. lessonii* (panel B) and *A. tepida* (panel C) embedded in resin and polished for Electron Probe Micro Analysis, the mapping area is depicted with a white box.



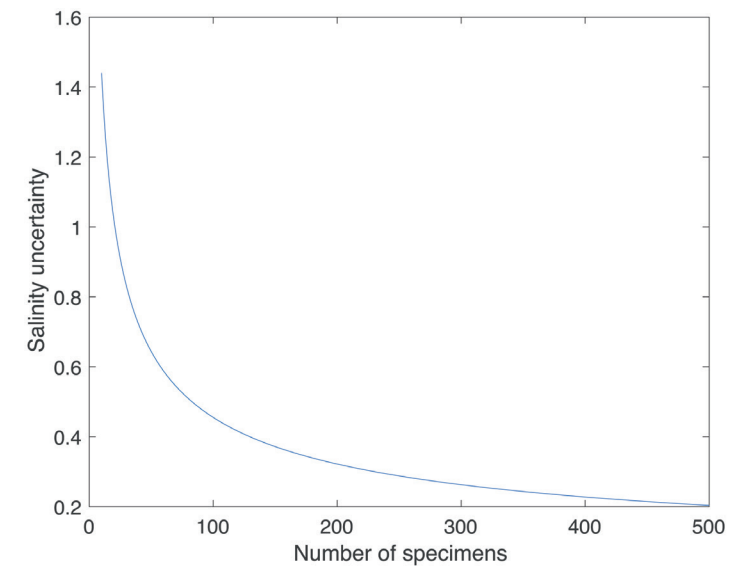
Appendix B. Results of the Monte Carlo analysis showing that the measured correlation coefficients for the inter-specimen correlations between the measured $El1/Ca_{cc}$ and $El2/Ca_{cc}$ are not caused by a spurious correlation due to the common denominator C_{acc} , showing that the measured correlation coefficient is significantly higher than the distribution of the correlation coefficients between 10,000 randomly drawn $El1$ concentrations/measured Ca concentration and measured $El2/Ca$ concentrations. This test is based on the concentration results from a single LA-ICP-MS session with specimens of *A. lessonii* cultured at a salinity of 35.

Appendix C. Results for the orthogonal regressions testing the correlations between single-spot $El1/Ca$ and $El2/Ca$, within salinity conditions, for *A. lessonii* and *A. tepida*.

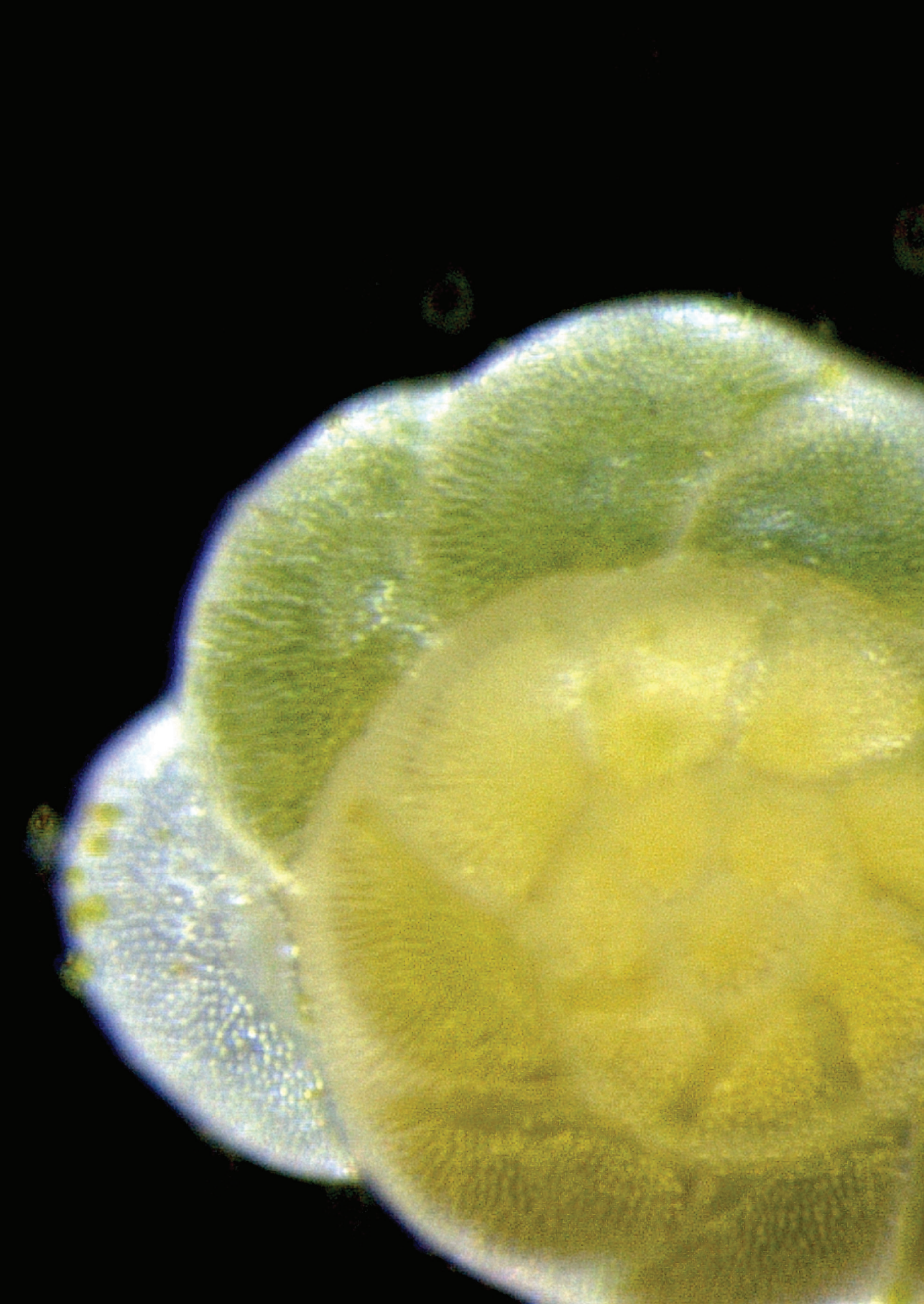
<i>A. lessonii</i>				<i>A. tepida</i>			
p-value (x, y)	r	Slope	y-Intercept	p-value (x,y)	r	Slope	y-Intercept
(Mg/Ca, Na/Ca)				(Mg/Ca, Na/Ca)			
p<0.001	0.71	0.21	2.21	p<0.001	0.35	1.64	1.63
p<0.001	0.52	0.17	3.89	p>0.05	0.24	2.14	0.41
p<0.001	0.57	0.17	4.06	p<0.001	0.62	0.86	3.14
p<0.001	0.89	0.14	5.44	p<0.001	0.53	1.19	2.35
p<0.001	0.69	0.16	5.12	p<0.001	0.84	1.34	1.89
(Mg/Ca, Sr/Ca)				(Mg/Ca, Sr/Ca)			
p<0.001	0.63	0.02	1.19	p<0.01	0.28	0.23	0.89
p<0.001	0.64	0.02	1.18	p>0.05	0.20	0.13	1.13
p<0.001	0.76	0.02	1.19	p>0.05	0.18	0.06	1.33
p<0.001	0.90	0.01	1.22	p>0.05	0.17	0.13	1.19
p<0.001	0.73	0.02	1.14	p>0.05	-0.28	-0.16	2.12
(Na/Ca, Sr/Ca)				(Na/Ca, Sr/Ca)			
p<0.001	0.58	0.09	1.00	p<0.01	0.29	0.14	0.66
p<0.001	0.47	0.10	0.78	p>0.05	0.10	0.06	1.10
p<0.001	0.72	0.10	0.77	p>0.05	0.23	0.07	1.10
p<0.001	0.94	0.11	0.62	p>0.05	0.18	0.11	0.94
p<0.001	0.80	0.12	0.53	p>0.05	-0.32	-0.12	2.34

A. lessonii

Appendix D. Foraminiferal Mg/Ca_{cc} and Na/Ca_{cc} (A and B, E and F) intensity ratio maps, obtained with EPMA, for two specimens of *A. lessonii* grown at a salinity of 30 (A-D) and 35 (E-H). Panel D and H show profiles for Mg/Ca (blue) and Na/Ca (red), based on averaged EPMA ratios scaled to LA-ICP-MS measurements in D and on EPMA count ratios in H (no LA-ICP-MS data available for this specimen), of an averaged transect area through the chamber wall perpendicular to the POS. The transect areas (purple rectangles) are indicated on top of backscatter SEM images (C and G), showing that the high El/Ca bands overlap with the primary organic sheet (POS, in dashed red line in C, not clear in G) and subsequent organic linings.



Appendix E. Figure showing the relationship between the salinity uncertainty and number of measured specimens for the Na/Ca_{cc} - salinity calibration of *A. lessonii*, calculated following as: $salinity\ uncertainty = (2 \times RSD \times \text{Number of specimens} - 0.5) / \text{sensitivity}$, whereby sensitivity is the slope of the calibration.



Chapter 3

Element banding and organic linings within chamber walls of two benthic foraminifera

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Geerken et al., 2019, Scientific reports, 9(1), 3598.

Image courtesy of Inge van Dijk

Abstract

Trace and minor elements incorporated in foraminiferal shells are among the most used proxies for reconstructing past environmental conditions. A prominent issue concerning these proxies is that the inter-specimen variability in element composition is often considerably larger than the variability associated with the environmental conditions for which the proxy is used. Within a shell of an individual specimen the trace and minor elements are distributed in the form of bands of higher and lower concentrations. It has been hypothesized that differences in specimen-specific element banding patterns cause the inter-specimen and inter-species variability observed in average element composition, thereby reducing the reliability of proxies. To test this hypothesis, we compare spatial distributions of Mg, Na, Sr, K, S, P and N within chamber walls of two benthic foraminiferal species (*Amphistegina lessonii* and *Ammonia tepida*) with largely different average Mg-content. For both species the selected specimens were grown at different temperatures and salinities to additionally assess how these parameters influence the element concentrations within the shell wall. Our results show that Mg, Na, Sr and K are co-located within shells, and occur in bands that coincide with organic linings but extend further into the calcite lamella. Changes in temperature or salinity modulate the element-banding pattern as a whole, with peak and trough heights co-varying rather than independently affected by these two environmental parameters. This means that independent changes in peak or trough height do not explain differences in average El/Ca between specimens. These results are used to evaluate and synthesize models of underlying mechanisms responsible for trace and minor element partitioning during calcification in foraminifera.

3.1 Introduction

Element incorporation into foraminiferal calcite provides a valuable tool for reconstructing seawater variables. For instance, foraminiferal Mg/Ca is a well-established proxy for seawater temperature (Anand et al., 2003; Elderfield and Ganssen, 2000; Lea et al., 1999; Nürnberg et al., 1996), whereas Na/Ca has recently been shown to reflect seawater salinity (Allen et al., 2016; Geerken et al., 2018; Mezger et al., 2016; Wit et al., 2013) and Ca-variability (Hauzer et al., 2018). Foraminiferal calibration studies show that a suite of other trace elements (e.g. U, Sr, Li and B), as well as fractionation of certain stable isotopes (e.g. $\delta^{11}\text{B}$) are related to parameters of the seawater carbonate system (Dawber and Tripathi, 2012; Hemming and Hanson, 1992; Keul et al., 2013b; Keul et al., 2017; Russell et al., 2004; Sanyal et al., 1996; Vigier et al., 2009), whereas Mn incorporation has been suggested to increase with decreasing bottom water oxygenation (Glock et al., 2012; Groeneveld and Filipsson, 2013; Koho et al., 2015; Koho et al., 2017; Petersen et al., 2018).

Comparison of these foraminiferal calibration studies with inorganic precipitation experiments (Lowenstam and Weiner, 1989; Pingitore Jr and Eastman, 1984, 1986; Weiner and Dove, 2003) shows that Element/Ca ratios (El/Ca) and the isotope composition of these elements in foraminiferal shells differ from those in inorganically precipitated calcite. In addition, there is considerable inter-species (Bentov and Erez, 2006; De Nooijer et al., 2017; Sadekov et al., 2014; Van Dijk et al., 2017b), inter-specimen (De Nooijer et al., 2014a; Sadekov et al., 2008) and intra-shell (Eggins et al., 2004; Fehrenbacher et al., 2017; Kunioka et al., 2006; Paris et al., 2014; Spero et al., 2015) variability in El/Ca ratios. The underlying biological mechanisms responsible for this variability and for the offset from inorganic-derived partition coefficients are not fully understood (Bentov and Erez, 2006; De Nooijer et al., 2014b; Erez, 2003; Marchitto et al., 2018). The proposed mechanisms include selective ion pumping (Bentov and Erez, 2006; Nehrke et al., 2013), pH regulation (De Nooijer et al., 2009b; Glas et al., 2012; Toyofuku et al., 2017), seawater vacuoles (Bentov and Erez, 2006), involvement of organic matrices (Branson et al., 2016), varying precipitation rates (Erez, 2003; Evans et al., 2015) and micro-environment effects (Holland et al., 2017; Wolf-Gladrow et al., 1999; Zeebe et al., 2003).

A key feature of foraminiferal geochemistry is systematic intra-shell chemical heterogeneity, which has the potential to inform us about the underlying mechanisms of test formation. This variability is most characteristically manifested in so-called banding, i.e., alternation of high and low concentrations of a given trace element in the direction parallel to the surface of the shell wall (Kunioka et al., 2006; Paris et al., 2014; Sadekov

et al., 2005; Spero et al., 2015; Steinhardt et al., 2015). Variability in the patterns of Mg banding, which is present in all species studied, has been hypothesized to be responsible for inter-specimen variability in Mg/Ca (Elderfield et al., 2002; Fehrenbacher et al., 2017; Sadekov et al., 2005; Spero et al., 2015). Furthermore, Mg banding is suggested to affect the reliability of the Mg/Ca-based paleo-thermometer, as differences in cleaning procedures between studies might alter the ratio between the amount of bands with high and low Mg concentrations and, thereby, the average Mg/Ca (Sadekov et al., 2005). The biological processes inducing Mg banding are hypothesized to be caused by temporal changes in the chemistry of the foraminiferal micro-environment during calcification. For example, Mg banding has been hypothesized to result from (i) diurnal oscillations in pH in the foraminiferal micro-environment as a function of symbiont activity, which is assumed to have a kinetic effect on Mg incorporation (Eggins et al., 2004; Lea et al., 1999), (ii) day-night patterns due to mitochondrial sequestration of Mg (Spero et al., 2015), or from (iii) alternating calcification pathways during chamber formation (Bentov and Erez, 2006; Erez, 2003). These factors may well be interacting and their relative contribution may vary between species, possibly explaining inter-species differences in Mg banding and average Mg/Ca values.

In addition to Mg, within-wall banding has also been observed for other elements, including Ba and Sr (Kunioka et al., 2006), Na (Branson et al., 2016; Geerken et al., 2018), S (Paris et al., 2014; Van Dijk et al., 2017a) and B (Allen et al., 2011; Branson et al., 2015). Variation in banding patterns for these elements may explain differences in average El/Ca values between specimens and species, although systematic studies of element banding in foraminiferal shells are scarce. Investigating how banding patterns differ between species and specimens grown at different conditions could enhance our understanding of the dependency of element incorporation on the environmental parameters. Furthermore, comparing element banding between different species and specimens could provide unique insights into the biological controls on element partitioning.

Here we systematically study the relationship between banding patterns and average El/Ca ratios in foraminiferal shells. Specifically, we test the hypothesis that differences in average El/Ca between specimens or species are due to either correlated or independent changes in El/Ca banding width or intensity across the shell wall. To this end, we used NanoSIMS (Nanoscale Secondary Ion Mass Spectrometry) to map the spatial distribution of Mg, Na, Sr and K in shells of two foraminiferal species with distinct Mg-content, *Amphistegina lessonii* and *Ammonia tepida* (~33 and ~3 mmol/mol Mg/Ca respectively (Geerken et al., 2018) grown under a range of salinities and temperatures. Although these two species are rarely used in paleoceanographic reconstructions, their Mg/Ca-

temperature sensitivities and absolute Mg/Ca span the complete range of sensitivities and absolute Mg/Ca known for Rotaliid species (Toyofuku et al., 2011; Wit et al., 2012). Investigating species that occupy the lower and upper ends of the spectrum increases the possibility to translate the results obtained here to other (paleoceanographically relevant) species. However, not all species may follow the same ontogenetic patterns as the species studied here, whereby new calcite lamella are added over previous whorls with every new chamber, yet some (e.g. *O. universa*, *N. dutertrei*), show daily calcite growth bands, apparently lacking organic linings in between (Fehrenbacher et al., 2017). Therefore, our additional aim is to investigate the spatial structure of the POS and of the subsequent organic linings, and specifically their relationship with the concentrations of Mg, Na, Sr and K across the shell wall. To this end, we used NanoSIMS to additionally measure the distribution of elements primarily associated with organic linings (Weiner and Erez, 1984), including N, S and P.

3.2 Methods

3.2.1 Sample selection and preparation

Specimens of *Amphistegina lessonii* and *Ammonia tepida* measured in this study were selected from previous culturing experiments conducted at different salinities (Geerken et al., 2018) and temperatures (van Dijk et al., 2019). Their average Mg/Ca, Na/Ca and Sr/Ca ratios, derived from measurements by Laser Ablation-Inductively Coupled Plasma-Mass Spectrometry (LA-ICP-MS) on 1–3 chambers, are listed in Table 1.

Table 1. Culture conditions and the corresponding single-specimen LA-ICP-MS obtained El/Ca ratios (mean ± SD) in the studied foraminiferal specimens.

Specimen code	Species	Salinity	Temperature (°C)	Na/Ca (mmol/mol)	Mg/Ca (mmol/mol)	Sr/Ca (mmol/mol)	n
1	<i>A. lessonii</i>	35	22	7.7±1.13	18.23±1.27	1.42±0.07	3
2	<i>A. lessonii</i>	35	29	8.12±0.75	28.07±3.18	1.73±0.22	3
3	<i>A. lessonii</i>	35	29	8.45±1.12	34.57±2.49	1.75±0.13	3
4	<i>A. lessonii</i>	30	25	7.69±0.2	29.5±0.99	1.86±0.04	2
5	<i>A. lessonii</i>	40	25	9.56±0.45	35.93±4.28	1.67±0.07	3
6	<i>A. lessonii</i>	40	25	9.8	29.2	1.81	1
7	<i>A. tepida</i>	25	25	2.94±0.04	2.07±0.18	1.18±0.04	3
8	<i>A. tepida</i>	25	25	3.48±0.03	2.04±0.12	1.26±0.05	2
9	<i>A. tepida</i>	35	25	3.90±0.22	1.50±0.21	1.45±0.05	2
10	<i>A. tepida</i>	40	25	4.29±0.32	3.39±0.27	1.40±0.03	2
11	<i>A. tepida</i>	40	25	4.19±0.13	2.71±0.27	1.41±0.06	3

After the LA-ICP-MS measurements, the specimens were embedded in an epoxy resin (Araldite 2020) in discs of 1 cm in diameter and 5 mm in height. This was done in a vacuum chamber to minimize entrapment of air bubbles within the foraminiferal chambers. After curing for 24 hours at 50 °C, specimens were polished using silicon carbide wet grinding papers with decreasing coarseness (HERMES, WS Flex 18C, 230 mm, P 800 and 219 ATM, SIC wet grinding paper, grain 4000). This resulted in a cross-sectioned sample with chamber walls exposed perpendicular to the shell walls, as evaluated by light microscopy. Exposed cross sections were subsequently fine-polished using agglomerated alpha alumina powder (Struers AP-A powder, grain size 0.3 µm) and SiO₂ powder (Logitech SF1 Polishing Suspension, grain size 0.035 µm). Finally, the polished samples were ultrasonically cleaned with ethanol, and coated with a 20 nm gold layer using a sputter coater (JEOL JFC-2300HR high resolution fine coater and JEOL FC-TM20 thickness controller).

3.2.2 Imaging by SEM, AFM and NanoSIMS

Imaging analyses by scanning electron microscopy (SEM), atomic force microscopy (AFM) and nanometer-scale secondary ion mass spectrometry (NanoSIMS) were performed at Utrecht University. SEM imaging was done with a JEOL Neoscope II JCM-6000 instrument using a backscattered electron detector. AFM imaging was performed with a Bruker Multimode III instrument operating in contact mode using silicon nitride tips on gold-coated cantilevers (Bruker Model SNP-10). Both height and deflection images were collected, and the measurements were done on the same areas as those measured by NanoSIMS and SEM. All images were collected in contact mode with a silicon tip attached to a triangular silicon nitride cantilever (spring constant 0.35 N/m, model SNL-10). Images were taken with a scan rate of 4.15 Hz with 384 lines per image. The instrument was calibrated prior to imaging using a standard grid with 200 nm pitch resulting in the precision in measured height of 2 nm.

SEM images were taken to identify areas suitable for NanoSIMS analysis and to locate organic linings, while AFM was used to analyze the sample surface topography after the NanoSIMS analysis. NanoSIMS analysis was performed with the CAMECA NanoSIMS 50L instrument. Using an element standard (SPI Supplies, 02757-AB 59 Metals & Minerals Standard), magnetic field and exact positions of the electron multiplier detectors were adjusted to enable detection of either positive (²³Na⁺, ²⁴Mg⁺, ³⁹K⁺, ⁴⁴Ca⁺, ⁸⁸Sr⁺) or negative (¹²C⁻, ¹⁶O⁻, ¹²C¹⁴N⁻, ³¹P⁻, ³²S⁻) secondary ions. The positive and negative secondary ions were detected using an 8 keV primary O⁻ and Cs⁺ ion source, respectively. First, areas of interest were pre-sputtered until secondary ion counts stabilized. The same pre-sputtering protocol was used on all samples to ensure comparability between different fields of view (FOV) measured. Subsequently, ion count images were acquired by rastering the

primary beam over the sample surface (areas between 8×8 and 40×40 µm in size) using the following diaphragm and slit settings: D0-2, D1-3, ES-2, AS-0 and EnS-0 for the O⁻ beam, and D0-0, D1-3, ES-2, AS-2 and EnS-1 for the Cs⁺ beam. These settings yielded a primary O⁻ beam at the sample surface of ~2 pA with a nominal size of 300-500 nm and a primary Cs⁺ beam of ~0.5 pA with a nominal size of ~100 nm, as defined and tested by CAMECA for the NanoSIMS instrument used. Additionally, the settings gave sufficient mass resolution to separate isobaric interferences while keeping high transmission. Secondary ions were detected with a dwell time of 2 ms/pixel (positive ions) and 0.8 ms/pixel (negative ions). To increase the overall signal the same FOV was imaged multiple times (400–1000), and the resulting ion count images were aligned and accumulated.

3.2.3 Data processing and analysis

Data processing was done using the freeware program Look@NanoSIMS (Polerecky et al., 2012) as well as additional custom-made routines in Matlab. First, effects of sample surface topography on the measured variability of elements within shells, were assessed by inspecting the overlay between the AFM and NanoSIMS images. Second, the spatial correlations between the organic sheets and the banding patterns seen in the distribution of elements were verified by overlaying the SEM and NanoSIMS images. Lateral profiles were drawn from the aligned SEM and NanoSIMS maps to allow comparison of the position of the organic linings.

The NanoSIMS images were aligned with the corresponding AFM or SEM images by manually adjusting the relative angle, displacement and magnification of one image against the other. This was done using a new interactive alignment tool added in Look@NanoSIMS. To ensure unbiased alignment these adjustments were made based on non-chemical features seen in the images, such as pores and edges of the calcite shell (Appendix A). Furthermore, this alignment strategy was compared with alignment of Ca-maps to SEM images of the NanoSIMS crater, which resulted in an indistinguishable alignment fundamentally independent from the first approach.

Further analysis involved lateral profiles of El/Ca (for positive ions) and El/O (for negative ions) ratios along lines oriented perpendicularly to the banding defined by organic sheets. When defining the lines, pixels close to edges, cracks or pores within the shells were avoided. Ten lateral profiles along lines parallel to the defined line were averaged to improve signal-to-noise ratio.

For each specimen, El/Ca ion count ratios obtained from the lateral profile were averaged and plotted against the corresponding average molar El/Ca ratio (Appendix B) derived

from the LA-ICP-MS analysis (Table 1). Since the two data sets showed good linear correlation when all specimens were combined (Appendix B), the regressions were used as calibration lines to convert the NanoSIMS-derived ion count ratios to molar El/Ca ratios in every pixel of the image (Table 2).

A typical lateral profile of El/Ca ratios within the foraminiferal shells comprised a succession of peaks and troughs. To allow comparison between specimens or different locations within the same specimen, we reduced each profile to a set of descriptors that included peak locations, peak width and height, and trough height. This was done in Matlab using a local peak finder function, which locates peaks for data points higher than its two neighbors and determines the peak prominence, i.e. the difference between the peak height and the two lowest neighboring values. A minimal acceptable peak prominence was set to avoid identification of noise in the data as false peaks. Peak and trough heights were defined, respectively, as the average height of the peaks and trough of the profiles.

The effect of the size of the NanoSIMS primary ion beam on the shape and width of the peaks in the lateral profiles of El/Ca and El/O ratios was assessed by calculating the spatial convolution between the Gaussian function, which represented the shape of the NanoSIMS beam, and an idealized distribution of the organic elements within the shell represented by a top-hat function (Branson et al., 2016). The width of the NanoSIMS beam was derived from the nominal beam size for the given instrument settings (full width half maximum (FWHM) of 355-640 nm for the O⁻ and 100 nm for the Cs⁺ beam), whereas the width of the top-hat function was assumed to be equal to the thickness of the organic linings derived from the SEM images (270nm).

Table 2. Results of linear regressions between the averaged ion count ratios obtained from NanoSIMS lateral profiles and the average molar El/Ca ratios obtained by LA-ICP-MS (Table 1). Mg/Ca were log-transformed for both the NanoSIMS and LA-ICP-MS data to account for the order of magnitude difference in Mg content between the two species. Na/Ca and Sr/Ca relationships were forced through zero. Note that the regressions are subject to some uncertainty as the areas analyzed by NanoSIMS were on different chambers than those ablated and analyzed by LA-ICP-MS.

El/Ca	Slope	SE slope	tStat	Intercept	R ²	p-Value
log(Mg/Ca)	0.91	0.06	14.77	-3.39	0.92	1.67E-11
Na/Ca	2.34E-01	2.45E-02	9.54		0.80	1.12E-08
Sr/Ca	2.38E-02	6.86E-04	34.68		0.005	1.21E-18

3.3 Results

3.3.1 Intra-shell element banding and organic linings

All SEM images of *A. lessonii* and *A. tepida* shells show thin brighter bands between calcite lamella (e.g. Fig. 1). These bands have been previously identified as organic linings (Hemleben et al., 1986; Ní Fhlaithearta et al., 2013; Weiner and Erez, 1984), and our NanoSIMS data corroborate this interpretation. Specifically, images of elements associated with organics (N and P) show thin bands at the same positions as the brighter bands in the SEM images (Fig. 1). Similar bands are also visible in images of S (Fig. 1), but this element can be associated with both organics (Weiner and Erez, 1984) and calcite (Paris et al., 2014). While S bands are pronounced in shells of both species, N and P bands are more pronounced in *A. tepida* (Appendix E). This difference between species is reflected also in the SEM images, which show brighter, thicker and hence more clearly visible bands for *A. tepida* (Fig. 1).

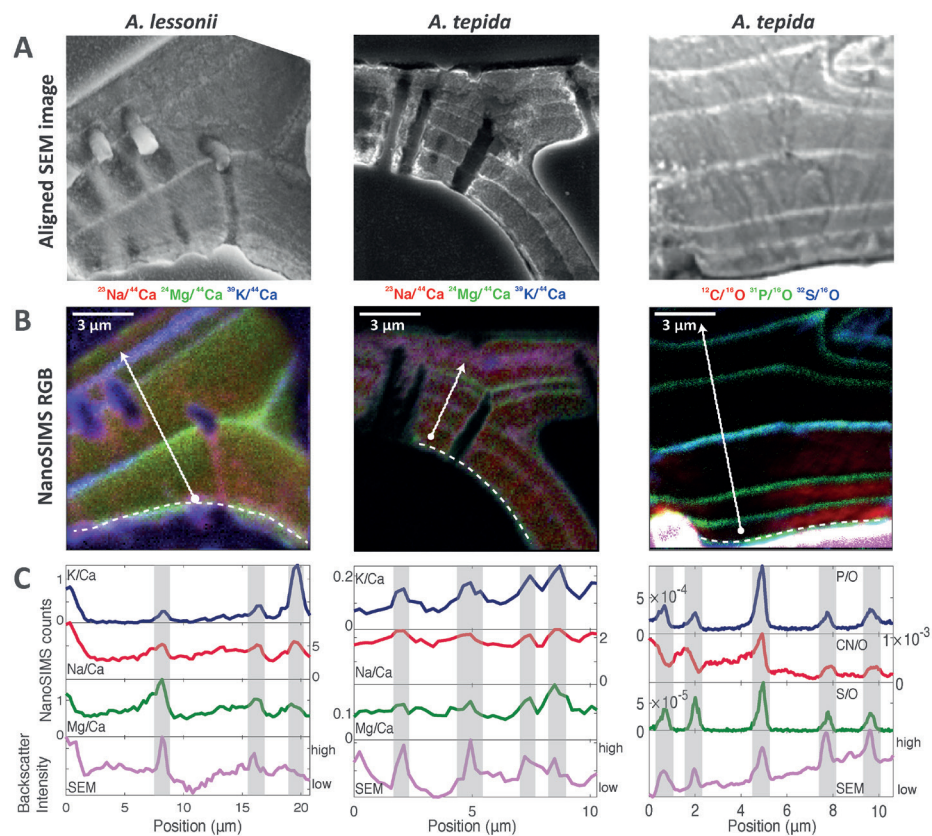


Figure 1. Comparison of backscattered electron images obtained with SEM and NanoSIMS RGB maps. A) Backscattered electron images, aligned to the NanoSIMS maps, showing the polished cross-sections of one specimen of *Amphistegina lessonii* (left column) and two of *Ammonia tepida* (middle and right) embedded in resin. B) NanoSIMS RGB maps showing spatial distributions of El/Ca in *A. lessonii* (left), *A. tepida* (middle) and El/O in *A. tepida* (right), corresponding to specimens #5, #9 and #11 (Fig. 3 and Table 1). Dashed white lines indicate the position of the Primary Organic Sheet, which appear similar in intensity to the other organic linings in the shell. C) Lateral profiles (white arrow indicated in panel B) through the aligned SEM images and NanoSIMS maps, showing the overlap between the brighter lines of the SEM image and peaks in high El/Ca and El/O .

Additionally, the AFM-derived topography images (Fig. 2) show that for *A. tepida* the organic linings are more elevated than the calcite lamella positioned between them, whereas for *A. lessonii* the linings are not clearly distinguishable (Fig. 2). Nevertheless, most of the topography observed, due to undulations in the surface around the pores, is not reflected in the NanoSIMS Me/Ca maps (Appendix E). This indicates that topography itself cannot entirely explain the observed El/Ca peaks around the organic linings. Furthermore, EPMA imaging, which is less sensitive to topography, on samples of the

same species shows similar geochemical banding patterns (Geerken et al., 2018; van Dijk et al., 2018). Calcium counts do not, in general, decline between lamella, suggesting that an edge effect on the Me/Ca due to pores or cracks is small (areas that did contain such cracks between lamella were excluded from further analysis).

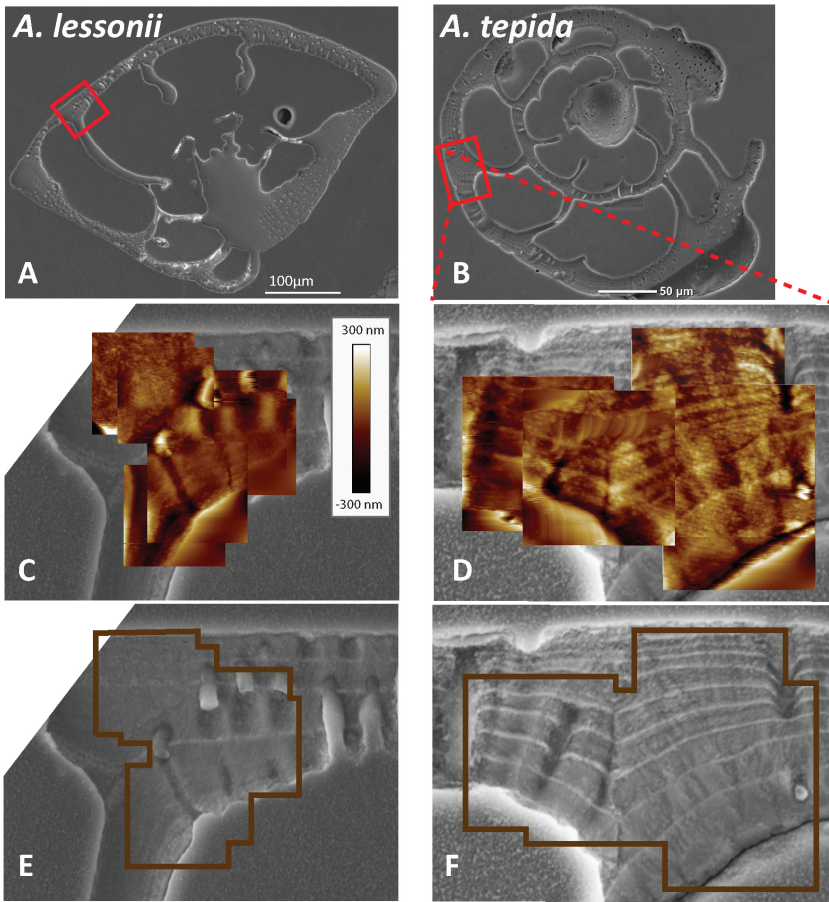


Figure 2. Overview SEM images of two specimens (A&B) and AFM height images superimposed on a close-up backscattered electron image (C&D), showing more pronounced topography related to organic linings in a specimen of *A. tepida* (A and C) than *A. lessonii* (B and D). The color scale bar is the same for both species. Note that there are some distortions in the *A. tepida* specimen that do not reflect height.

For both species the distributions of Mg, Na and K show clear banding patterns (Fig. 3). Close inspection of the overlays between the NanoSIMS and SEM images and of the corresponding lateral profiles shows that the peaks of these metals are found at the location of the organic linings (Fig. 1). Thus Mg, Na and K are spatially linked with the

organic linings in the shells of the studied species. Sr is more homogeneously distributed than the other metals, however it does seem to show a banding pattern as well (Fig. 3). Calcium counts do not, in general, decline between lamella, suggesting that an edge effect on the El/Ca due to pores or cracks is small. Analysis of all available SEM and NanoSIMS image pairs revealed no apparent systematic differences when comparing the primary organic sheet (POS) with subsequent organic linings. Therefore we henceforth refer to them collectively as ‘organic linings’.

Although spatially linked, there is a systematic difference between the bands of the “organic elements” (N, P and S) and the studied metals. While the widths of the organic element bands are about 280 ± 50 nm, the metal bands are significantly broader (810 ± 160 nm for *A. tepida* and 1160 ± 280 nm for *A. lessonii*). Numerical analysis revealed that this difference cannot be explained by the larger size of the O^- beam used for the measurements of the metals in comparison to the Cs^+ beam used for the measurements of the organic elements (Appendix D). Thus the metal peaks in the studied shells are broader than the organic linings. The widths of the organic element bands are about 2–3 fold larger than the previously reported values of 100–130 nm (Branson et al., 2016), but this difference is likely due to the presence of a nanometer-scale branched organic 3D-structure surrounding the sheet (Branson et al., 2016), falling within this width.

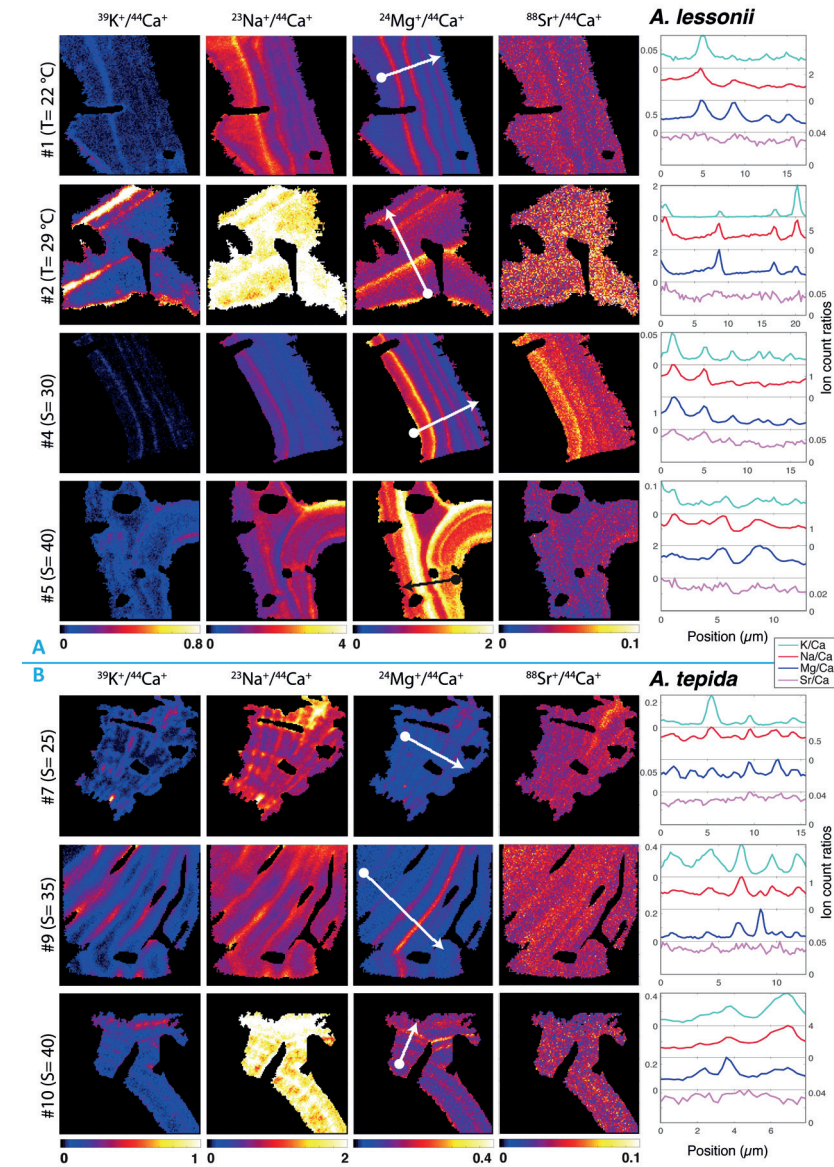


Figure 3. Examples of representative NanoSIMS count ratio maps of K/Ca, Mg/Ca, Na/Ca and Sr/Ca (left to right) of *Amphistegina lessonii* specimens #1, #2, #4 and #5 (panel A) and *Ammonia tepida* specimens #7, #9 and #10 (panel B) for the salinity experiment (S=25, S=30, S=35, and S=40) and temperature experiment (T=22 °C and T=29 °C). Specimen numbers correspond to specimen ID in Table 1. Color map scales of El/Ca maps are the same between specimens to facilitate comparison between maps and measured LA-ICP-MS El/Ca values. Lateral profiles (right panel) show that El/Ca peaks generally coincide and are asymmetrical in *A. lessonii*. Profiles, with a width of 10 pixels, are indicated with white arrows showing the direction from inside to outside of the shell wall (Mg/Ca panel).

3.3.2 Intra-shell element distribution: variability between species and specimens

Although the two species studied here have about 10-fold different average Mg/Ca ratios, their element banding patterns are generally similar. In both species, metal bands are parallel to the inner- and outer surface of the chamber walls, following the lamellar structure of Rotaliid chamber formation. The main difference between *A. tepida* and *A. lessonii* is that both peaks and troughs for Mg/Ca are about 10-fold higher (Fig. 3) and peaks are about 1.4 broader in *A. lessonii* compared to *A. tepida* (Appendix D). Furthermore, relationships between Na/Ca and Sr/Ca versus Mg/Ca along the lateral profiles show steeper slopes in *A. tepida* (Appendix Table B1, Appendix F). Additionally, metal peaks in *A. lessonii* are more asymmetrical, increasing towards the outside of the lamella in most maps (e.g., specimens #2 and #4) while declining towards the outside in others (e.g., specimen #1), especially for Na/Ca (Fig. 3). In contrast, metal peaks in *A. tepida* are more symmetrical and narrower, hence more closely confined to the organic linings separating the lamella, especially for K/Ca (Fig. 3).

Both the NanoSIMS and LA-ICP-MS data (Geerken et al., 2018) show that there is considerable variability between specimens with respect to their El/Ca lateral profile and average El/Ca values, irrespective whether they have been grown in different or the same environmental conditions. Interestingly, an increase in the average El/Ca ratio, as measured by LA-ICP-MS, is related to an increase of both peak and trough height of the lateral profiles, as measured by NanoSIMS (Fig. 4, Appendix B). In contrast, peak widths do not show a significant relationship to the mean El/Ca values. In both species the distance between peaks is determined by the width of the lamella, which increases with chamber number. Peak distance would therefore systematically affect inter-specimen differences in El/Ca only if lamellas became thinner with higher El/Ca, which is not observed. Together, these observations show that inter-specimen variability, between specimens from the same or different conditions, is not explained by independent changes of the peak or trough height, but is driven by a proportional increase of the entire profile (Fig. 4, Appendix B).

For the *A. lessonii* specimens studied here, increased average Mg/Ca, Na/Ca and Sr/Ca in specimens grown at a higher temperature or higher salinity results from a proportional increase of both peaks and trough heights (Fig. 4). It has to be stressed, however, that our study does not reflect the response to environmental conditions at a population level, and we refer to van Dijk et al. 2019 for results showing temperature effect on Sr/Ca, Mg/Ca and absence of an effect of temperature on Na/Ca. Our study shows, however, that El/Ca peak heights are ~2 times higher than El/Ca trough heights (see S2 for regression results), and that the entire within-shell element distribution of an *A.*

lessonii specimen is thus elevated with increasing average-shell El/Ca values (Fig. 4 and Appendix B). For *A. tepida*, El/Ca peak height shows a significant linear relationship with El/Ca trough height, with a slope of 1.4 and intercept of 0.7 (see Appendix B for regression results). Both Na/Ca peak and trough height are increased in specimens grown at higher salinities, however, the trend is not fully proportional, since the peak height intercept is positive (Fig. 4).

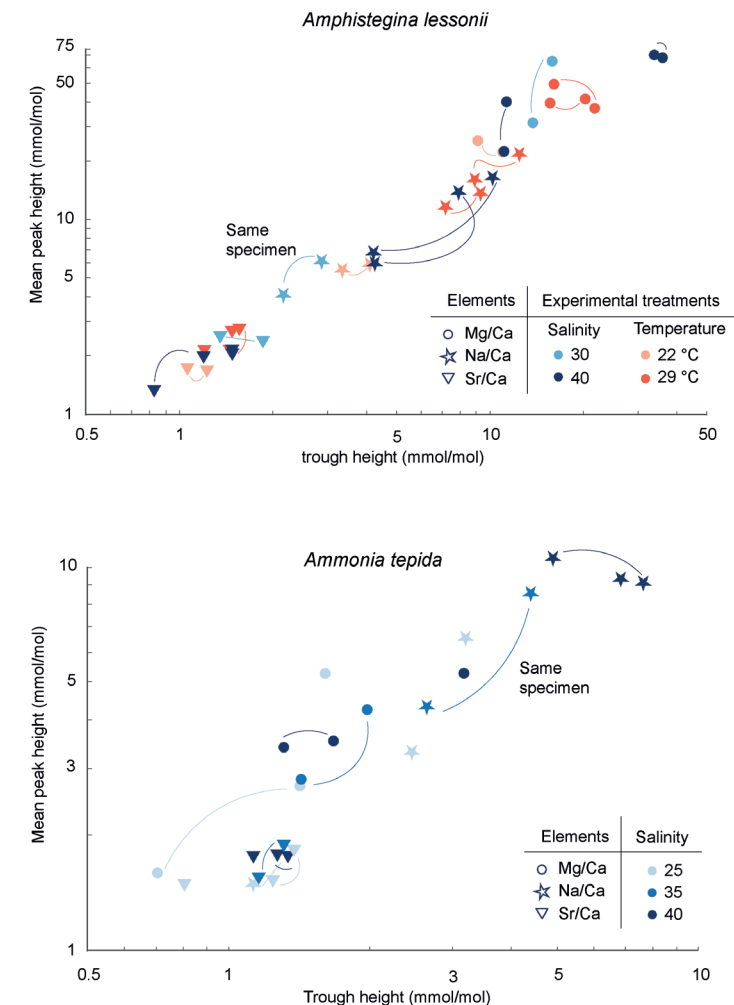


Figure 4. Average peak height versus trough height for Sr/Ca (triangles), Na/Ca (stars) and Mg/Ca (circles) based on lateral profiles (see Appendix B for regression results). Every symbol represents one NanoSIMS map: two maps from the same specimen are connected with a line. Both peak and trough values increase (proportionally) with increasing average El/Ca values, resulting either from environmental effects (e.g. higher Mg/Ca with increasing temperature) or inter-specimen variability (grown in the same environmental conditions yet different average El/Ca values).

3.4 Discussion

3.4.1 Role of organic linings in calcification and the relationship with element banding

Organic linings play a fundamental role during the first phase of carbonate precipitation in foraminifera, by providing a template upon which calcification occurs (Hemleben et al., 1986). However, the organic template of many organisms (e.g. oysters, mollusks) likely provides more than just structural support (Addadi et al., 2006; Addadi and Weiner, 1985; Ní Fhlaithearta et al., 2013). Organic linings in foraminiferal shells have been shown to be spatially (Kunioka et al., 2006) or even mechanistically associated to trace and minor element bands in some species, where the primary organic sheet (POS) is suggested to adsorb cations (Branson et al., 2016). This POS is formed by a dense pseudopodial network prior to calcification, and gives the new chamber its overall shape (Hemleben et al., 1977; Spero, 1988). Furthermore, organic linings are suggested to have a branched structure in *O. universa* rather than a laminar sheet structure (Branson et al., 2016). Organic linings differ in composition and characteristics between species, with *A. tepida* showing organic linings that are more resistant to decalcification compared to those of other species (Ní Fhlaithearta et al., 2013). This is in line with our SEM and AFM results, showing brighter and more elevated organic linings in *A. tepida* than in *A. lessonii* (Figs. 1 and 2). Furthermore, this difference in the organic linings of both species is also reflected in the NanoSIMS maps, which show more pronounced P and N peaks in *A. tepida* than in *A. lessonii* (S4, Appendix E). Possibly, these different characteristics of the organic linings are related to the difference in Mg/Ca content between species, for example by affecting Mg uptake or by controlling nucleation (e.g. by determining the CaCO_3 phase formed).

Elevated signals of N, P and S in our NanoSIMS maps (Fig. 1, Appendix E) likely originate from organic compounds such as proteins and sulfated polysaccharides that are present in organic linings of foraminifera (Weiner and Erez, 1984). Sulfated and acidic groups of these compounds are hypothesized to help overcome the free energy barrier prohibiting crystal nucleation and guide crystal growth (Addadi and Weiner, 1985; Weiner and Dove, 2003; Weiner and Erez, 1984). P peaks are broader (~270 nm) than previous estimations of organic lining thickness of ~100-130 nm (Branson et al., 2016), which could be due to the presence of a smaller-scale 3D structure within the P peak width. These structures have been observed in *O. universa* with Transmission Electron Microscopy (Branson et al., 2016), yet cannot be resolved within the spatial resolution of our Cs-source maps (~100 nm). Within the calcite lamella, P ion counts are low (between 0-2 counts, 100 times lower than in the organic linings), suggesting that the organic linings and a surrounding small scale 3D structure around the sheets of *A.*

tepida and *A. lessonii* is confined to a small region (~300 nm) rather than a substantial branched structure extending into the calcite lamella (~5 µm). However, since P ion counts do not entirely decline to zero (1-2 counts), single organic macromolecules might be present at small concentrations, within the calcite or between the calcite rods, as we cannot detect how ions are incorporated. Single organic macromolecules are observed in other biomineralizing species such as mollusks (Weber and Pokroy, 2015), and have been suggested to increase the hardness of the carbonate shells (Kim et al., 2016).

Sulfur peaks observed in our NanoSIMS maps can be indicative of the sulfated groups of the organic compounds that form the organic linings (Weiner and Erez, 1984). Although S-peak widths are similar to P-peak widths (both ~270 nm), S counts are ~10 times higher than P counts, and S counts do not decline to zero in between the peaks (10-15 counts, Fig. 1, Appendix E). Furthermore, in some specimens S peaks show shoulders (asymmetrical) within the lamella between the organic linings (Fig. 1, Appendix E). This could be sulfur in the form of calcite-bound SO_4^{2-} (Paris et al., 2014), yet our study cannot resolve between calcite or organic hosted sulfur. Furthermore, organic-bound elements potentially have different ionization efficiencies, making it difficult to directly compare relative S/O-ion counts between the organic and calcite fractions. However, previous studies suggest that the calcite-bound SO_4^{2-} fraction is higher than the organic-bound S fraction (Paris et al., 2014; Van Dijk et al., 2017a).

Our finding that El/Ca peaks are co-located with organic linings (Fig. 1) is in line with other studies on intra-specimen element distribution in Rotallid (benthic) foraminifera (Erez, 2003; Geerken et al., 2018; Kunioka et al., 2006; Nakajima et al., 2016; Van Dijk et al., 2017a). An Atom Probe Tomography-Time-of-flight-Secondary Ionization Mass Spectrometry study showed that the interface of the organic template in *O. universa* is enriched in Na and Mg (Branson et al., 2016), suggesting that metals other than Ca^{2+} might be bound to charged functional groups, or that incorporation of these elements is increased due to lattice strain induced by the organic template. It has been suggested that organic-bound Na contributes significantly to Na-variability within the shell, and hence may affect the paleo-application of this novel proxy (Branson et al., 2016). Adsorption to the organic template may well explain part of the observed El/Ca peaks, especially in *A. tepida*, showing peaks that are closely positioned around the organic linings. However, our measurements show that the El/Ca peaks, particularly in *A. lessonii* profiles, are significantly broader than organic elements peaks (Appendix D). Furthermore, a recent study using X-ray spectroscopy demonstrated that part of the Na is most likely calcite-bound in foraminifera shells (Yoshimura et al., 2017). This is corroborated by the relatively high Na/Ca-troughs in our NanoSIMS maps (Fig. 3),

suggesting that there is considerable Na-variability in both *A. tepida* and *A. lessonii* calcite, which is not associated to high levels of organic compounds. However this study cannot resolve whether Na is structurally bound in the calcite or present between the rods. K/Ca peaks appear to be more strictly confined to the organic template and decline to near zero values in the lamella (Fig. 3), resembling the spatial distribution of organic elements. Since metal peaks are broader than the organic element peaks, and peak heights are related to trough heights for all elements, the calcification mechanism employed by foraminifera might play a role in the banding patterns, which is discussed in more detail in section 3.4.3.

3.4.2 Environmental effects on element banding and inter-specimen variability; implications for proxies

The first reports on intra-shell Mg heterogeneity (Eggins et al., 2003; Eggins et al., 2004) questioned the reliability of the Mg-paleothermometer. More recent studies have aimed to reconcile this large variability in composition with an overall and consistent increase in Mg/Ca with temperature, for example by showing that both low- and high-Mg concentration bands increase with temperature (Fehrenbacher et al., 2017; Spero et al., 2015). In our dataset, an increase in average Mg/Ca, both between species and between specimens, regardless of environmental condition, is accompanied by a proportional increase in both Mg/Ca peak and trough height. Mg/Ca peak height is hence systematically related to trough height, whereby the relative intra-shell variability remains similar (Fig. 4). NanoSIMS profiles have been used previously to examine Mg/Ca ratios taken from single specimens transferred to higher temperatures, allowing the authors to circumvent potential offsets due to inter-specimen variability (Fehrenbacher et al., 2017). Our increasing Mg/Ca peaks and troughs observed in *A. lessonii* specimens grown at higher temperature (Fig. 4) fit well with the lateral profiles of single specimens in the previous study, indicating that the proportional increase is applicable both within and between specimens and is depending on temperature. However, in contrast to our findings, a study using depth-resolved LA-ICP-MS measurements shows that a similar, rather than a proportional, increase in both low- and high-concentration Mg-bands, occurs in specimens of *O. universa* grown at increasing temperatures (Spero et al., 2015). This difference may result from differences between species (or groups: e.g. benthic versus planktic) or between experimental and methodological treatments (e.g. NanoSIMS in this study versus LA-ICP-MS profiles (Spero et al., 2015). Temperature seems hence to exert an overall effect on the trough as well as peak Mg concentrations through thermodynamic principles, kinetic effects, affecting the Mg uptake mechanism by the organism or a combination of these effects (Branson et al., 2013). Our NanoSIMS analysis and results from previous studies (Fehrenbacher et al., 2017; Spero et al., 2015) increase confidence in

the Mg-paleothermometer, as the overall element's spatial distribution is systematically raised (Fig. 4). This appears to justify a working model on foraminiferal Mg/Ca based on the observation that Mg is incorporated in a uniform crystallization mechanism (Branson et al., 2013). It has to be noted however that the latter study (Branson et al., 2013) did not capture the primary organic sheet itself, so part of the Mg peak might still be adsorbed to the organics. Future research should hence look at the incorporation of elements into the calcite in the vicinity of organic templates, within ~300 nm.

The incorporation of Na in foraminiferal shells has been shown to depend on salinity (Geerken et al., 2018; Mezger et al., 2016; Wit et al., 2013) and seawater Na/Ca (Hauzer et al., 2018). This could be explained by an effect of ionic strength on the Na activity or partition coefficient (Wit et al., 2013). Na/Ca peaks and trough heights increase with increasing salinity, proportionally in *A. lessonii* and almost proportional in *A. tepida* (Figs. 3 and 4), although the limited number of NanoSIMS maps does not allow for robust statistical testing. The correspondence between the positive peak intercept in *A. tepida* (Fig. 4) for Mg and Na with the organic linings may be explained by a relative large contribution of adsorbed Mg and Na expected in the organic linings (Branson et al., 2016). This might influence the paleo-application of Na/Ca to reconstruct salinity, since degradation of the organic linings and adsorbed Na might alter the average Na/Ca over time (Branson et al., 2016). Furthermore, it has been suggested that also structurally bound Na is leached due to burial diagenesis (Yoshimura et al., 2017), which further complicates the down-core application of Na/Ca.

Sr/Ca banding in foraminiferal shell walls is generally less pronounced than that of Mg/Ca, K/Ca or Na/Ca (Fig. 3). Subtle changes in Sr/Ca were also observed within the test wall of *Pulleniatina obliquiloculata*, with high ion counts coinciding with the high Mg-bands (Kunioka et al., 2006). The fact that Sr/Ca banding is (much) less pronounced than that observed for Na/Ca and Mg/Ca may also be explained by the Sr counts being low in both species. In *A. lessonii*, Sr/Ca trough and peak heights increase with temperature (Fig. 4). This is in line with van (van Dijk et al., 2019) showing that, in addition to increasing Mg partition coefficients, Sr partition coefficients also increase with increasing temperature. This suggests that in high-Mg species, Sr concentration is not only affected by a carbonate ion effect (Keul et al., 2017) but also by temperature. This effect could be direct or indirect, e.g. through Mg induced differences in lattice strain, which enhances Sr incorporation (Evans et al., 2015; Mucci and Morse, 1983). This is consistent with our results, showing that Mg/Ca and Sr/Ca are strongly correlated along the lateral profiles. In *A. tepida*, Sr/Ca banding is less clearly pronounced (fig. 3).

The K/Ca ion counts within test walls show a strong correlation with Na/Ca ion counts (Appendix F, Appendix Table B1), and both K/Ca minima and maxima increase with salinity, although K/Ca peaks are highest at salinity 35 for *A. tepida*. Since Na/Ca has been shown to correlate with salinity, a similar within-shell wall behavior of K/Ca suggests that also this element might have the potential for constraining past salinities, although application will depend on a suitable analytical approach to accurately and precisely determine K/Ca.

Altogether, these results show that element banding itself, e.g. uncorrelated changes in peak or trough height, does not explain observed differences in El/Ca among specimens as a function of e.g. temperature, salinity or inter-specimen variability (Fig. 4, Appendix B. and Fig. 5). The simultaneous increase in peak and trough height across elements (Fig. 4) hints to a single mechanism responsible for the intra-shell variability in Mg/Ca, Sr/Ca and Na/Ca, although it remains to be investigated what this control is. Our results confirm that calibrating El/Ca to environmental conditions using LA-ICP-MS data requires careful inspection of the profiles for completeness, e.g. covering the entire thickness of the shell wall.

3.4.3 Implications for biomineralization

Element banding in foraminiferal shells most likely reflects biomineralization related processes. Banding is most pronounced for elements under tight biological control such as Mg (De Nooijer et al., 2014b; Zeebe et al., 2001). Several biomineralization mechanisms have been invoked to explain the element bands in planktic foraminifera, such as differences in symbiont activity (Eggins et al., 2004; Hathorne et al., 2009; Sadekov et al., 2005), changes in Mg flux to control calcite precipitation (Jonkers et al., 2016) or induced by diurnal light-dark cycles (Fehrenbacher et al., 2017; Spero et al., 2015).

Elevated concentrations of Mg, Na, Sr and K coincide with the position of organic linings, suggesting that incorporation of these elements is relatively high during the initial phase of the formation of a calcite layer or that they are potentially associated with the organic lining. In addition, concentrations of the metals analyzed here in some specimens (e.g. specimen #2) also (gradually) increase towards the outside of an individual lamella, indicating that also the final stages of the formation of an individual calcite lamella are accompanied by a weaker elemental discrimination compared to seawater El/Ca, especially in *A. lessonii*. This observation could be explained by Rayleigh fractionation (Elderfield et al., 1996), whereby elements are incorporated from a biomineralization reservoir with element-specific organic partition coefficients. With ongoing calcification, the Ca-fraction in the reservoir is reduced by precipitation of CaCO_3 and elements with

an organic partition coefficient <1 (such as for Sr, Mg and Na) will hence increase towards the end calcification (Elderfield et al., 1996). However, recent studies suggested that Ca is taken up from the seawater during calcification (Marchitto et al., 2018; Nehrke et al., 2013), either through Ca-pumps, channels or vacuoles enriched in Ca, which would (partially) counter-act the Rayleigh fractionation effect. Alternatively, changes in element concentrations within a lamella might also be related to variability in e.g. the precipitation rate (Elderfield et al., 1996), Ca-influx or pH at the site of calcification. In addition, the tight control on leakage of trace element during calcification may decrease towards the end of a calcification event so that the fluid from which calcite is precipitated starts to resemble seawater and foraminiferal partition coefficients are closer to those reported for inorganically precipitated calcite.

Co-location of element bands (Hathorne et al., 2009; Kunioka et al., 2006, this study; Paris et al., 2014), likely relates to the reported inter-element correlations between species (De Nooijer et al., 2017; Evans et al., 2015; Van Dijk et al., 2017b) and between specimens (Geerken et al., 2018; Mewes et al., 2015). There are several hypotheses that provide an explanation for co-located element banding that will be discussed to first test their applicability to our and previously published results and, second, to evaluate potential environmental controls (i.e. temperature and salinity) on element incorporation and banding therein.

1) *Dual-phase calcification*. It was proposed that the formation of every individual lamella is a two-step process: an initial high Mg-phase is precipitated close to the organic lining, followed by an additional low-Mg phase (Erez, 2003). These two phases are thought to be formed by smaller and larger spherulites, consisting of high- and low-Mg calcite, respectively, as observed in decalcified specimens of *A. lobifera*. Changes in the relative contribution of these high Mg-phases (for instance due to a higher temperature) could be responsible for the observed increase in average Mg/Ca with temperature (Bentov and Erez, 2006). Formation of the high-Mg spherulites was further hypothesized to invoke secondary calcification by reducing the Mg concentration within the site of calcification (SOC). These first smaller spherulites would also be enriched in P, which together with Mg^{2+} is the major inhibitor for calcite nucleation and growth in seawater (Morse et al., 2007). Such a different crystallographic phase may affect the incorporation of other elements as well, which is in line with the general correlation between all elements observed in our study. Our NanoSIMS data showing El/Ca peaks around the organic linings and troughs in between (Fig. 3) appears to confirm the existence of the suggested different phases (Bentov and Erez, 2006). However, we also show that the entire intra-shell El/Ca lateral profiles distribution (both peak and trough height) shifts towards

higher El/Ca ion counts for specimens (and species) with higher mean El/Ca values (Fig. 4). This is in contrast to the suggested increase in the relative contribution of the high-Mg phase explaining variability in Mg/Ca between specimens (Bentov and Erez, 2006; Erez, 2003). Furthermore, a uniform crystallographic Mg coordination in both high- and low-concentration bands (Branson et al., 2013) suggests that the two types of bands only differ in their Mg-content, but are otherwise crystallographically similar. Together, this questions the dual-phase crystallization pathway as the primary mechanism for the observed inter-species variability in Mg/Ca and other El/Ca ratios.

2) *Adsorption to organic molecules.* Surface adsorption of cations to negatively charged functional groups at the interface of the organic matrix (Branson et al., 2016) could explain the observed coupled increase of cations near the organic linings. Observed banding for P and N, which most likely reflects the actual organic material, is confined to the organic linings as observed with SEM, than the broader bands of the metals measured here (Fig. 1, Appendix A). As these smaller metal ions can be accommodated in the calcite structurally, this could explain the gradual decline away from the organic lining (Fig. 3) versus the sharp peaks of organic elements (Appendix E), probably incorporated as PO_4 and NO_3 or amino acids, that are incompatible with the calcite structure. Note that we cannot distinguish between organic and calcite-bound ions, and that there could be a matrix effect, which could produce apparent higher counts during the NanoSIMS analysis. Some of the metals that produce the observed peaks, might hence be adsorbed to organic compounds, which for Na might compromise its use as a salinity proxy (Mezger et al., 2016). In *A. tepida*, Na adsorption at the interface of the organic lining could explain the relative increase of the trough compared to peak height with increasing salinity. Still, Na/Ca troughs appear unrelated to large organic structures (>100nm) and future studies should focus on the structural position and crystallographic coordination of Na.

Organic linings can also introduce lattice strain, which could explain enhanced and coupled element incorporation around the organic linings. Recent studies have suggested that D_{Sr} increases with increasing Mg content due to the effect of Mg-induced lattice strain, between species (Evans et al., 2015) and between specimens of *A. lessonii* (Mewes et al., 2015). The relationship between Mg/Ca and D_{Sr} in hyaline species (Evans et al., 2015; Van Dijk et al., 2017b) and in *A. lessonii* specimens (Geerken et al., 2018; Mewes et al., 2015) closely resembles the relationship for inorganically precipitated calcite (Mucci and Morse, 1983). As Mg disrupts the calcite crystal structure by introducing lattice strain, its incorporation should also affect the partition coefficients of other elements incorporated into the calcite structure, such as Na (Yoshimura et al., 2017) and Sr (Finch

and Allison, 2007). An Mg-induced lattice strain effect could hence also explain co-varying Sr and Na with Mg bands in *A. lessonii* similar to inorganic precipitation studies. At low Mg levels it is unlikely that Sr and Na will be significantly impacted by Mg-induced lattice strain. Hence, the 8-10 times steeper inter-element slope for *A. tepida*, at the intra-shell and inter-specimen level, hints at a higher partition coefficient. However, inorganic precipitation experiments are required to quantify the effect of increasing Mg-content on Na incorporation, as well as how coupled effects of D_{Mg} due to lattice strain may be altered by the formation of precursor phases such as vaterite and ACC.

3) *Modes of ion transport to the site of calcification.* Abiotic precipitation experiments from artificial seawater result in calcites with high Mg/Ca ratios compared to those in foraminifera. The organism hence must be able to lower Mg/Ca ratios relative to seawater Mg/Ca. Currently, the mechanisms by which Mg and Ca (and other ions) reach the site of calcification in foraminifera is heavily debated. Below, we discuss how the proposed mechanisms could or could not explain our observations.

The two transport models that have gained relatively high attention are the ‘trans-membrane transport’ (TMT) model (Nehrke et al., 2013) and seawater vacuolization model (Erez, 2003). Both models rely on selective ion transport to create the low-Mg/Ca solution from which calcification commences in foraminifera (De Nooijer et al., 2014b). In the TMT model, selective Ca-transport to the site of calcification (SOC) occurs in combination with ‘passive transport’ of seawater leaking from the environment into the SOC. Alternatively, in the second model vacuolized seawater undergoes selective ion (i.e. Mg^{2+}) removal before the release of a low-Mg/Ca fluid into the SOC (Bentov and Erez, 2006; Erez, 2003). In addition to these two biomineralization models, ions (and their isotopes) may undergo additional partitioning and fractionation due to processes operating during calcite precipitation, e.g. Rayleigh fractionation (Elderfield et al., 1996) and lattice strain effects (Evans et al., 2018), which will be discussed later. Our results cannot distinguish between these types of ion transport, but both TMT and vacuolization are used to describe the change in ion supply over time and its potential in explaining element banding.

In the both biomineralization models based on ion transport, variation of El/Ca within the shell wall could result from changes in the elemental composition of the SOC (e.g. the El/Ca ratio) and/or changes in the inorganic partition coefficients (e.g. D_{Mg}), as expressed by the following equation:

$$\text{El/Ca}_{(t)} = D_{\text{El}} \times \frac{\text{El}_{\text{SOC}}}{\text{Ca}_{\text{SOC}}}$$

D_{El} is often estimated from inorganic precipitation experiments in which precipitation usually proceeds through classical crystal growth (Mucci and Morse, 1983; Tesoriero and Pankow, 1996). Element incorporation in these studies is shown to be affected by pH of the seawater, which should therefore be taken into account when translating inorganic partition coefficient to partitioning in the SOC during calcification (Elderfield et al., 1996; Erez, 2003; Evans et al., 2015; Marchitto et al., 2018; Nehrke et al., 2013).

Species with very low average Mg/Ca, like *Ammonia tepida*, but also planktic species often used in paleo-applications, may be characterized by a SOC that is well-separated from the surrounding seawater (Nagai et al., 2018b). Ions necessary for calcification may enter the calcifying fluid by selective inward Ca^{2+} trans-membrane transport (TMT) enclosing the site of calcification (Bentov and Erez, 2006; Erez, 2003; Nehrke et al., 2013; Toyofuku et al., 2017) or by Ca^{2+} from vacuolized seawater (Erez, 2003). Both the increase of the pH (due to proton pumping) and the higher Ca^{2+} concentration at the SOC will facilitate $CaCO_3$ nucleation and growth by increasing the saturation of the fluid present with respect to calcite. Inward Ca^{2+} -transport is then assumed to be the dominant source of Ca in the SOC and hence determines (the low) El/Ca of the fluid from which the calcite precipitates. Foraminiferal species precipitating their calcite from an open, more seawater-like, SOC, or often replenished biomineralization space (Elderfield et al., 1996), will show El/Ca values close to ratios reported in inorganic precipitation experiments from seawater. A fluid with a El/Ca resembling seawater might derive ions from seawater vacuoles, which have been observed in higher Mg species such as *Amphistegina lobifera* (Evans et al., 2018). Higher fractions of unmodified seawater and lower Ca^{2+} -transport (both increasing the El/Ca ratio towards seawater El/Ca) could both explain why all El/Ca ratios measured here are higher in the intermediate Mg species *A. lessonii* compared to low-Mg species *A. tepida*.

Variability in the relative contributions of selectively transported Ca^{2+} and seawater/modified vacuoles might hence not only explain differences in El/Ca (co-) variability at the inter-species level (Nehrke et al., 2013), but also explain differences in El/Ca between specimens. Variable Ca^{2+} transport during the chamber calcification, could explain intra-specimen El/Ca variability (e.g. element banding) if Ca^{2+} levels are in- and decreasing during chamber formation. Alternatively, hypothesized outward Mg^{2+} -transport (Bentov and Erez, 2006) would also be reflected in El/Ca banding perpendicular to the growth direction of the shell. As selective removal of Mg^{2+} (Bentov and Erez, 2006; Erez, 2003) would have a dominant effect on Mg-banding and only a limited effect on banding of other elements (with possibly the exception of Sr and Na in high-Mg species) it is more likely that Ca-pumping or passive transport variability is responsible for the synchronous banding in all metals.

Ca-transport models may thus explain the observed inter-elemental correlations within the shell (Appendix Table B1, Appendix F), between specimens (Geerken et al., 2018) and between hyaline foraminifera (De Nooijer et al., 2017; Evans et al., 2015; Van Dijk et al., 2017a; Van Dijk et al., 2017b). This is further corroborated by the observation that the inter-element trend for *A. lessonii* specimens and for hyaline foraminiferal species approximately falls into the line between $El/Ca=zero$ and $El/Ca_{seawater}$ ratios times the inorganic D_{El} 's (albeit that the intercept for Na and Sr is >0 , which could be explained by the additional impact of adsorbed cations at the organic linings).

The slopes of the intra-shell Mg/Ca-Na/Ca and Mg/Ca-Sr/Ca relationships are respectively 8 and 10 times higher for *A. tepida* than for *A. lessonii* (Appendix F; Appendix B). The same increase in slopes is observed for the inter-specimen relationship between these elements (Geerken et al., 2018). Hence, our NanoSIMS observations on intra-specimen combined with inter-specimen inter-element correlations (Geerken et al., 2018) suggest that there must be a parameter affecting element incorporation that differs between the two species studied here. This could be related to changes in the partition coefficient D_{Na} , between species (and potentially, over time), which then should be about 8 times higher in *A. tepida* compared to *A. lessonii*. The partition coefficient, in turn, could be affected by calcite growth rates, which is likely affected by the pH and $[Ca^{2+}]$ at the SOC (Mucci and Morse, 1983; Tesoriero and Pankow, 1996). A difference in precipitation rates between species could, for example, explain a higher D_{Na} in *A. tepida* compared to *A. lessonii* (Busenberg and Plummer, 1985), although this hypothesis requires future testing. Additionally, a higher Mg^{2+} concentration in *A. lessonii* could slow down precipitation rates by increasing mineral solubility (Davis et al., 2000).

Phase transformations during calcite precipitation are another variable affecting element partitioning. Recently, a vaterite precursor phase has been identified in several foraminiferal species (Jacob et al., 2017), implying inorganic partition coefficients might have to be reassessed since the partition coefficients in step-wise calcite growth may differ from partitioning through a vaterite precursor. This process has been suggested to also allow for low Mg values without the need of complex ion-transport models, by means of (double) fractionation against Mg through phase-transitions from ACC to vaterite (not observed yet but hypothesized) and vaterite to calcite (Jacob et al., 2017). It could be imagined that species with higher Mg, such as *A. lessonii*, a direct phase transformation from ACC to calcite, due to the presence of Mg, explains the difference between low and slightly elevated Mg levels (hence through a single or double fractionation step). Irrespective of the $CaCO_3$ phase(s) involved, ion incorporation may be also be affected by Rayleigh fractionation during the formation of a chamber, which could explain the

increase in El/Ca towards the end of the lamella as observed in *A. lessonii* specimens (Fig. 3).

If calcite precipitation takes place in a (semi-) enclosed environment, differences between ions in incorporation rates result in a change in the ratios between these ions in the SOC due to Rayleigh fractionation effects (Allen et al., 2011; Bentov and Erez, 2006; De Nooijer et al., 2014b; Erez, 2003; Nagai et al., 2018b; Toyofuku et al., 2017). This, in turn increasingly affects the element composition of the layers precipitated subsequently, resulting in increasing or decreasing element concentrations over time (Elderfield et al., 1996; Evans et al., 2018; Marchitto et al., 2018). Such a process could additionally affect element concentrations and if the mode of ion transport into the SOC (either by TMT or vacuoles) changes over time, would give rise to a pattern of alternating high- and low El/Ca bands. In theory, the combination of all processes mentioned above operate in concert and are likely required to fully explain element banding and its variability across species.

3.5 Conclusions

Despite their different average El/Ca ratios, specimens of both the benthic foraminifers *Amphistegina lessonii* and *Ammonia tepida* have chamber walls that show clear element banding patterns for Mg, Na, Sr, K, S, P and N. Peak locations overlap for all elements measured here and coincide with the location of the organic linings separating the calcite lamellae of the shell walls. Peak widths of S and P, associated to the organic linings, are significantly smaller than El/Ca peak widths, especially in *A. lessonii*. The 10-fold difference in average Mg/Ca (~33 mmol/mol in *A. lessonii* versus ~3 mmol/mol in *A. tepida*) corresponds to a ~10 fold increase in peak and trough height of Mg/Ca in *A. lessonii* compared to *A. tepida* (Fig. 5). Furthermore, we show that the entire within-shell wall El/Ca -distribution is raised in specimens with higher El/Ca average values. Hence, peak and trough heights show a proportional (in *A. lessonii*) or linear (in *A. tepida*) relationship between specimens, whether grown in different conditions or the same conditions and reflecting inter-specimen variability in El/Ca (Fig. 5). This observation enhances confidence in proxies based on element incorporation in foraminiferal calcite, as the ratio between low- and high-concentration bands is not affected by the environmental condition. The observed 8-fold increase in Mg/Ca - Na/Ca slopes between *A. tepida* and *A. lessonii* intra-specimen trends in our NanoSIMS maps and at the inter-specimen level (Geerken et al., 2018) hint at a difference in the calcification pathway between these species.

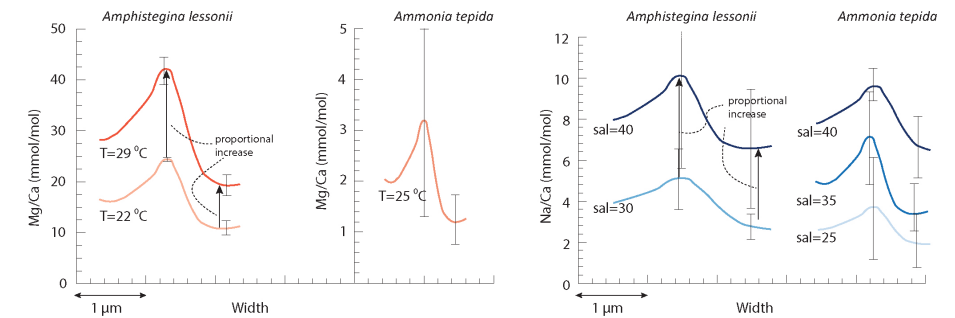
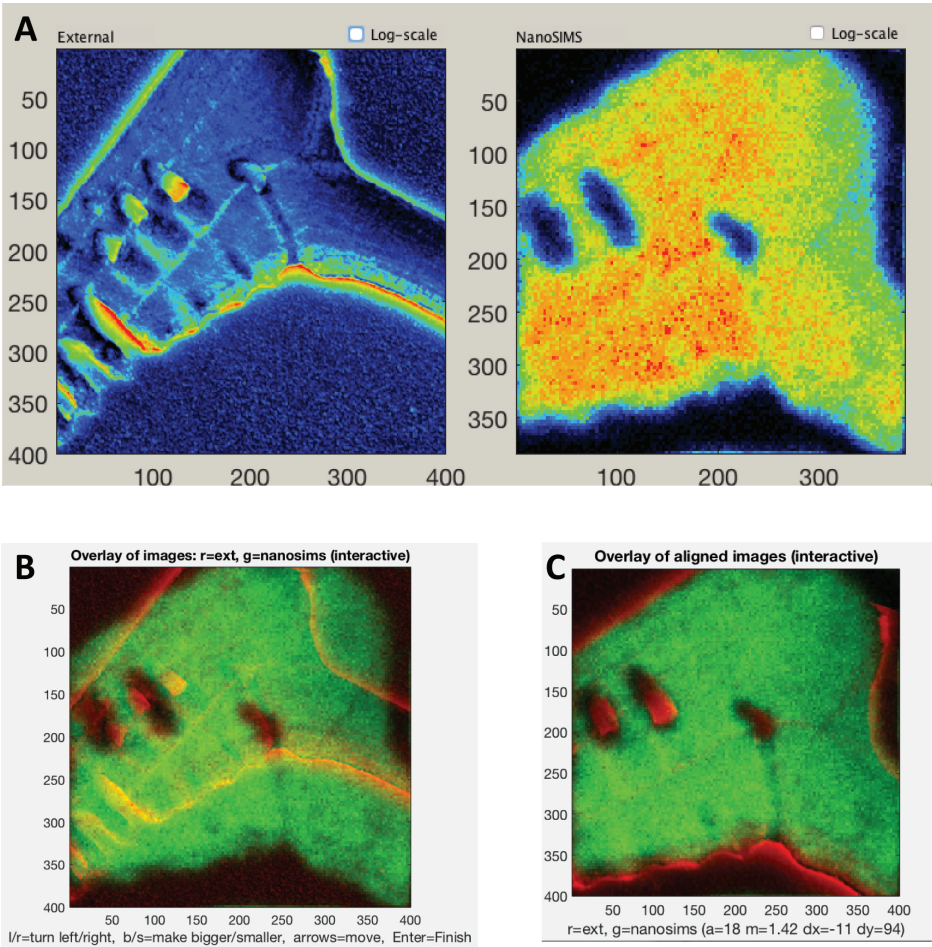


Figure 5. Illustration of the average element banding patterns with environmental conditions, based on the average El/Ca peak and trough height per condition, showing the proportional increase in Mg/Ca peak and trough height between species and between *A. lessonii* specimens grown in different temperatures (left). The effect of salinity (right) on Na banding in *A. lessonii* and *A. tepida* is a linear increase of the trough and peak height, whereby the trough is increasing relatively more than the peak height. Error bars indicate ± 1 SD surrounding the mean peak and mean trough values. The position of the error bars is sometimes offset along the x-axis for clarity and for the Na/Ca of *A. lessonii* grown at a salinity of 40, the ± 1 SD has been partly removed.

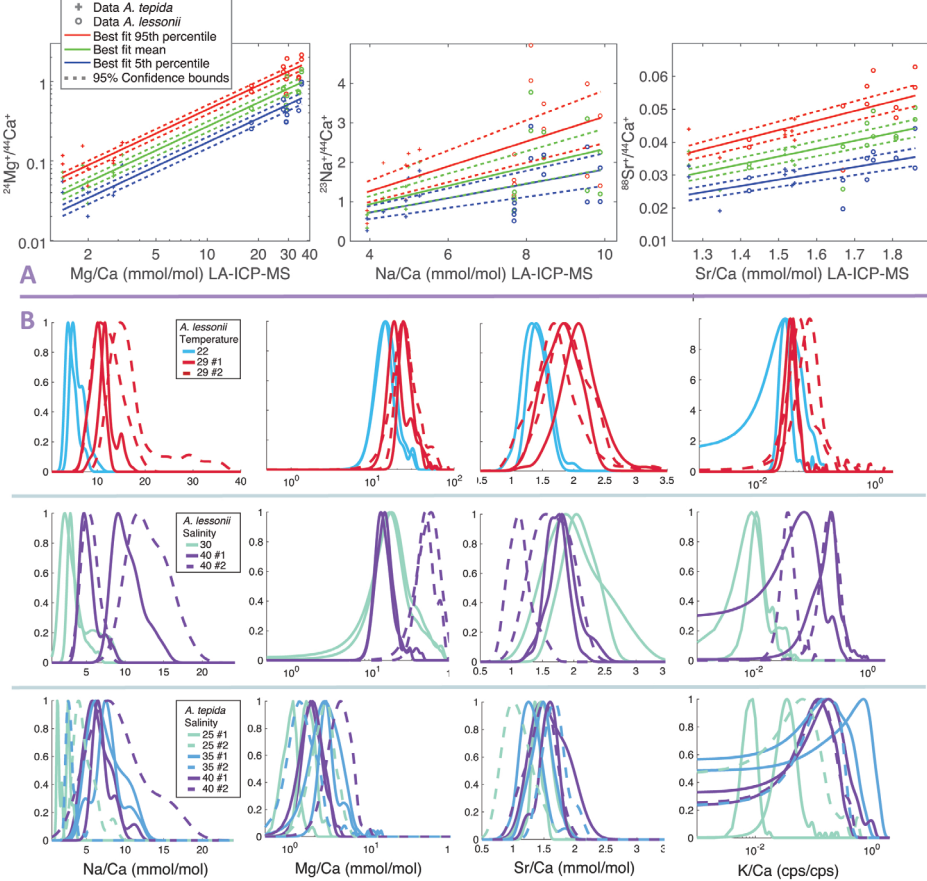
Acknowledgements

We thank Inge van Dijk for providing foraminiferal specimens from the temperature experiment, Michiel Kienhuis for technical support with NanoSIMS analyses, and Wim Noorduyn and Anat Akiva for fruitful discussions. The NanoSIMS facility at Utrecht University was financed through a large infrastructure grant by the Netherlands Organisation for Scientific Research (NWO) awarded to Jack Middelburg and Gert-Jan Reichart (grant no. 175.010.2009.011). This work was carried out under the programme of the Netherlands Earth System Science Centre (NESSC), financially supported by the Ministry of Education, Culture and Science (OCW) (grant no. 024.002.001).

Appendix



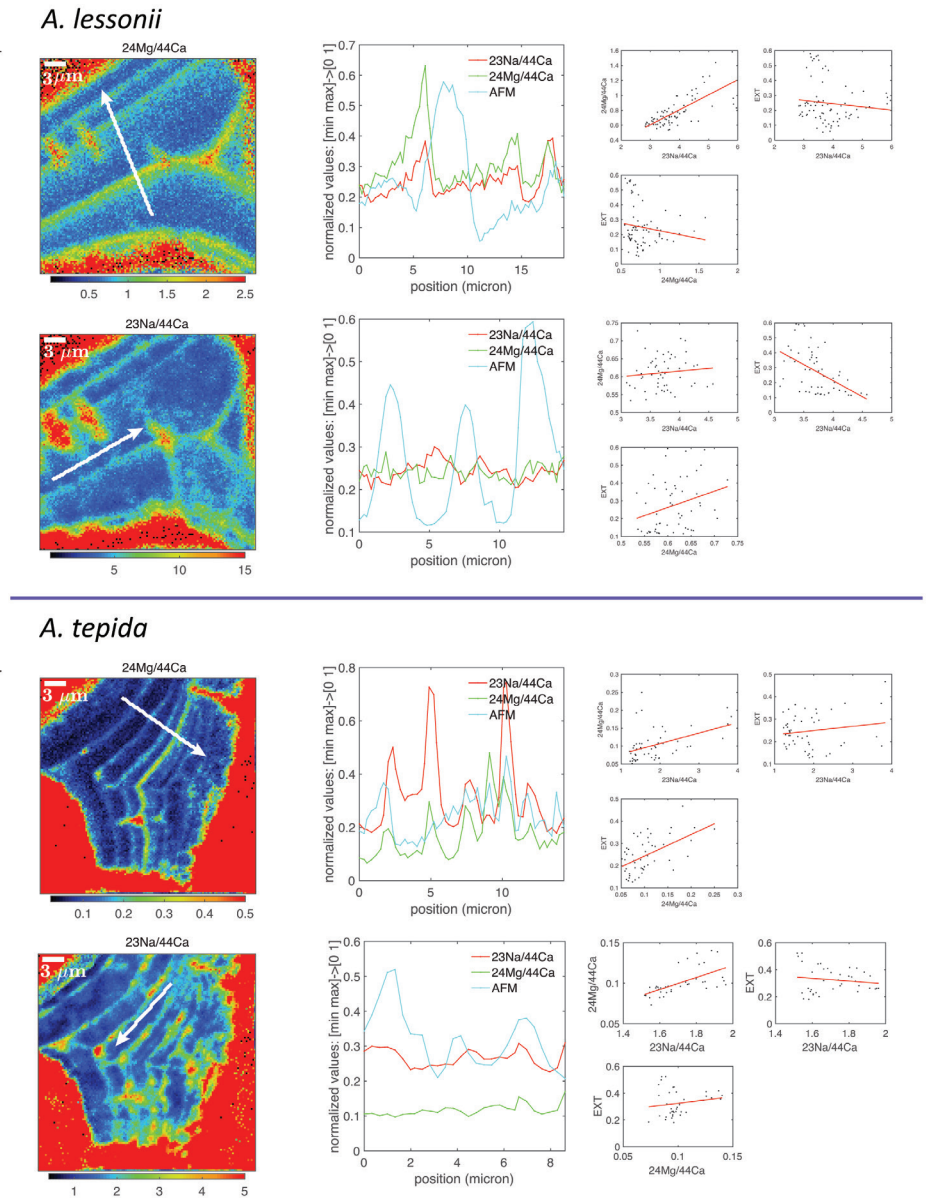
Appendix A. Example of the alignment of a SEM image to a NanoSIMS Ca-counts map using the interactive alignment tool in Look@NanoSIMS. SEM and AFM images were aligned to NanoSIMS maps using the Look@NanoSIMS Interactive Alignment Tool. This tool enables rotation, zooming and shifting of an external image, here a SEM image (left in panel A, red in panel B and C) to align it with a NanoSIMS map, here a Ca counts map (right in panel A, green in panel B and C). Panel C shows the final alignment, which is still within a few pixels error.



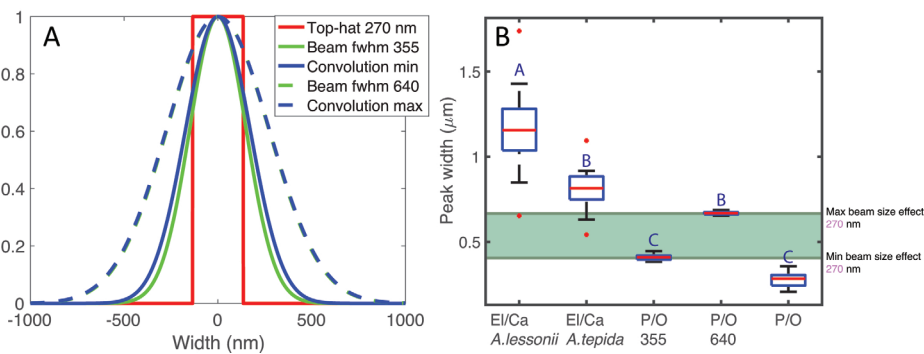
Appendix B. Panel A: Maximum, mean and minimum count ratios for Mg/Ca, Na/Ca and Sr/Ca based on NanoSIMS maps obtained for *A. tepida* and *A. lessonii* (in red the 95th percentile, in green the mean and in blue the 5th percentile ion counts of the lateral profiles), versus individual average LA-ICP-MS El/Ca values, with the 95% confidence bounds for the fitted coefficients in dashed lines. The relationship between the mean count ratios and LA-ICP-MS El/Ca values is used for the calibration of ion counts to molar ratios, see Table 2 for regression results. Panel B: distribution plots for Na/Ca, Mg/Ca, Sr/Ca and K/Ca, showing the density distributions of El/Ca values observed within the lateral profiles, color-coded per environmental condition. Dashed/non-dashed lines indicate whether lateral profiles are from different maps on the same specimen. The top row shows the *A. lessonii* specimens from the temperature experiment, the middle row specimens of *A. lessonii* from the salinity experiment and bottom row those of *A. tepida* from the salinity experiment. The entire intra-shell spatial distributions shift for specimens grown in different conditions. Within temperature and salinity conditions, different specimens also show a shift in the density distributions, whereas in most cases, density distributions of different maps on the same specimen are similar.

Table B1. Orthogonal regression results of lateral profiles for Mg-Na, Mg-Sr and K-Na for *A. tepida* and *A. lessonii* specimens grown under different experimental conditions. *Sal* stands for salinity experiment and *Temp* for temperature experiment, see Table 1 for details. See Appendix F for scatter plots and regression lines.

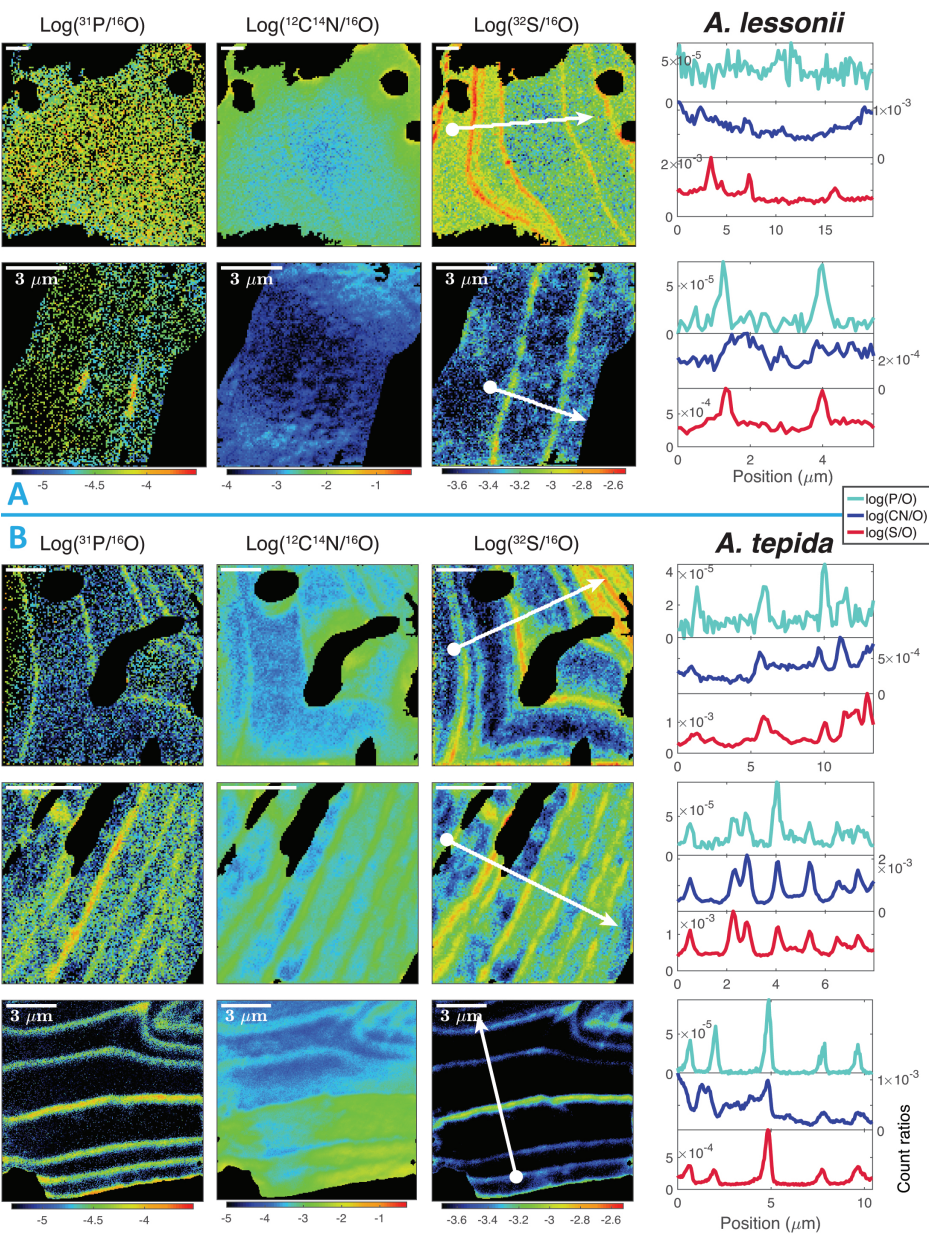
<i>A. lessonii</i>	Experimental condition	Slope	Intercept	R	SD Slope	SD Intercept
Mg-Na	Sal 30	0.67	0.34	0.93	4.46E-02	4.12E-02
	Sal 40	1.56	0.73	0.71	1.43E-01	8.07E-02
	Temp 22	1.63	1.22	0.79	1.33E-01	9.09E-02
	Temp 29	1.74	1.04	0.83	1.38E-01	1.51E-01
Mg-Sr	Sal 30	0.02	0.03	0.69	2.69E-03	2.25E-03
	Sal 40	0.03	0.02	0.45	3.96E-03	2.20E-03
	Temp 22	0.02	0.03	0.56	2.81E-03	1.83E-03
	Temp 29	0.02	0.02	0.54	2.95E-03	3.18E-03
K-Na	Sal 30	36.56	0.31	0.74	3.95E+00	6.70E-02
	Sal 40	14.16	0.95	0.54	1.46E+00	7.09E-02
	Temp 22	23.25	1.15	0.85	2.20E+00	9.68E-02
	Temp 29	11.70	1.57	0.74	1.23E+00	1.26E-01
<i>A. tepida</i>	Experimental condition	Slope	Intercept	R	SD Slope	SD Intercept
Mg-Na	Sal 25	8.40	0.18	0.67	1.36E+00	8.40E-02
	Sal 35	11.87	0.29	0.81	1.41E+00	1.01E-01
	Sal 40	14.08	0.76	0.56	2.28E+00	1.79E-01
Mg-Sr	Sal 25	0.23	0.02	0.59	4.31E-02	2.16E-03
	Sal 35	0.27	0.02	0.33	5.39E-02	3.74E-03
	Sal 40	0.22	0.02	0.47	4.05E-02	3.55E-03
K-Na	Sal 25	20.57	0.37	0.65	3.28E+00	5.30E-02
	Sal 35	1.10	0.78	0.88	9.52E-02	3.08E-02
	Sal 40	2.9	1.27	0.77	3.23E-01	7.81E-02



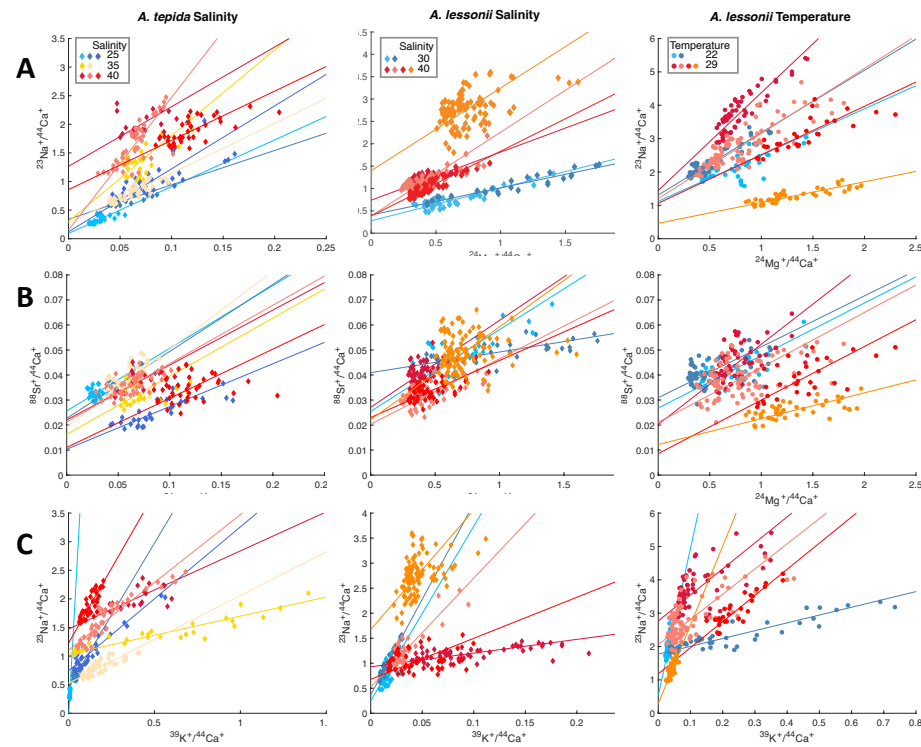
Appendix C. Comparison of AFM and NanoSIMS maps showing that much of the observed topography is not reflected in the ion count ratios. The organic linings in *A. tepida* occasionally appear to be elevated in the AFM profiles, whereas those in *A. lessonii* are not detectable. Since most topography, due to undulations in the surface within the lamella, is not reflected in the NanoSIMS maps, we assume this is co-variation rather than a causal relationship.



Appendix D. A). Convolutions (in green) of a top-hat function (red) of 270 nm (average ‘organic’ El/O peak width as measured with the Cs-source) and Gaussian functions (blue) with a FWHM (full width at half maximum) based on adjusted Python script by Branson et al. (2016). The Gaussian FWHM is based on the nominal O-beam size expected with our settings as measured by Cameca, expected to be ~300nm with a maximum of 500 nm, which is converted to FWHM as 355 (solid lines) and 640 nm (dashed lines). B) Boxplots showing the FWHM peak width distributions of *A. lessonii* and *A. tepida* El/Ca (Sr/Ca, Mg/Ca and Na/Ca) and P/O peak width distributions, measured with the Cs-source with a smaller nominal beam size (100nm), adjusted for the O-beam size effect, with 355nm being the expected beam size and 640nm the maximum beam size. Letters above the boxplot show whether distributions are significantly different. For the expected beam size of 355nm, both *A. lessonii* and *A. tepida* peak width distributions are significantly different from the beam-size corrected P/O peak widths distributions. For the maximum beam size of 640 nm, only *A. lessonii* peak widths are significantly different from the beam-size corrected P/O peak widths distribution.



Appendix E. Examples of representative NanoSIMS maps of S, P, C (ratio to O) for specimens of *A. lessonii* (panel A) and *A. tepida* (panel B). Lateral profiles, indicated by a white arrow from the inside to the outside of the shell, showing clear peaks at the position of the organic linings separating the calcite lamella (Fig. 1). Color bars are the same for each element to enhance comparability between maps. All scale bars are 3 μm.



Appendix F. Scatterplots of the lateral profiles drawn from NanoSIMS count ratio maps, with orthogonal regression lines showing the relationship between elements within the shell-wall. Lateral profiles from all specimens, grown in different conditions (color-coded), are significantly correlated for Mg/Ca versus Na/Ca (A), Mg/Ca versus Sr/Ca (B) and K/Ca versus Na/Ca (C) for *A. tepida* (left) and *A. lessonii* (middle=salinity, right=temperature). See Appendix B for regression results.



Chapter 4

High precipitation rates characterize biomineralization in foraminifera

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Abstract

The chemical composition of foraminiferal calcite reflects seawater variables and is therefore a popular tool to reconstruct past environments and – climates. However, the relationship between calcite chemistry and environment is imprinted by cellular controls during biomineralization. Kinetic and thermodynamics processes at the site of calcification likely govern precipitation rate and hence element partitioning, but are under tight biological control. Here we determine calcite precipitation rates in the low-Mg foraminifer *Ammonia beccarii* by labelling the growing shell wall and subsequent imaging of the label using NanoSIMS. We show that precipitation rates are surprisingly uniform between specimens, at $\sim 22 \pm 2$ nmol/cm²/min, comparable to the highest rates obtained by inorganic calcite precipitation experiments. Moreover, the amount of Sr incorporated in foraminifera can be explained as a function of precipitation rate for precipitation from a Mg-free, seawater-like solution. The first layer of calcite is precipitated at a significantly lower rate and enriched in Mg²⁺, SO₄²⁻ and Na⁺ compared to the rest of the shell wall. The initial calcite is hence likely precipitated from a seawater-like fluid, while subsequent layers are deposited from a calcifying fluid resupplied by Ca²⁺ and Sr²⁺ via trans-membrane transporters. These results aid the understanding of the role of precipitation rate (i.e. kinetics) on trace element partitioning and element banding in foraminifera and thereby, improve the reliability of proxies based on foraminiferal shell chemistry as a paleoceanographic reconstruction tool.

4.1 Introduction

Calcium carbonate is the most widely occurring biomineral on Earth (Knoll, 2003), with a profound impact on global carbon cycling and atmospheric carbon dioxide concentrations (Ridgwell, 2005; Ridgwell and Zeebe, 2005; Zeebe and Westbroek, 2003). Foraminifera, unicellular marine organisms that evolved 500 million years ago (Culver, 1991; Pawlowski et al., 2003), secrete calcite shells that form approximately 50% of the open ocean calcium carbonate (Schiebel, 2002). Besides their impact on the global carbon cycle, the chemical composition of fossil specimens is a frequently used tool to reconstruct past environments and climates (Anand et al., 2003; Lea et al., 1999). This is possible since incorporation of trace elements and fractionation of isotopes during biomineralization depend on environmental conditions, including temperature (Lea et al., 1999; Nürnberg et al., 1996; Rosenthal et al., 2000), salinity (Geerken et al., 2018; Mezger et al., 2016; Urey et al., 1951; Wit et al., 2013), element composition (Boyle, 1981) and seawater pH (Sanyal et al., 1996). These relationships can be partly explained by kinetic and thermodynamic principles, but also reflect a tight biological control during calcification (Erez, 2003; Urey et al., 1951). For most trace elements, concentrations in the calcite shell are relatively low compared to non-biological calcite precipitated from seawater, which has fuelled debate on the mechanisms responsible for transporting ions from seawater into the calcifying space (De Nooijer et al., 2014b; Elderfield et al., 1996; Erez, 2003; Marchitto et al., 2018; Nehrke et al., 2013; Toyofuku et al., 2017). Identifying the mechanisms involved in foraminiferal biomineralization is essential for understanding element partitioning between seawater and foraminiferal shell carbonate and hence their applicability for reconstructing past environments.

Observations on biomineralizing foraminifera show the involvement of seawater vacuoles (Bentov and Erez, 2006; Erez, 2003; Evans et al., 2015), pH-regulation (Bentov et al., 2009; De Nooijer et al., 2009b; Toyofuku et al., 2017), organic templates (Branson et al., 2016), membranes secluding the calcifying fluid from seawater, and complex cytoskeleton arrangements (Tyszka et al., 2019). Together, these processes determine the conditions and element composition of the calcifying fluid from which foraminifera precipitate their calcite. Within this confined space, kinetic and thermodynamic effects determine element partitioning and hence the eventual composition of the calcite shell. Environmental conditions (e.g. temperature or salinity) likely affect element incorporation through these kinetic or thermodynamic factors, but may also influence the biological controls on calcite precipitation. Efforts to integrate these various effects on element incorporation resulted in models including Rayleigh fractionation (Elderfield et al., 1996), specific ion (e.g. Mg²⁺) removal (Bentov and Erez, 2006) and trans-membrane Ca²⁺-transport into

the calcifying fluid (Nehrke et al., 2013). Although these models are able to explain part of the observed sensitivities of element incorporation to environmental parameters, a large unknown with a profound impact on foraminiferal shell chemistry is the calcite crystal precipitation rate, which remains poorly quantified.

Precipitation rate has been shown to impact element partitioning in inorganic calcites^{30–34}, whereby higher rates promote incorporation of Na^+ (Busenberg and Plummer, 1985), B^+ (Mavromatis et al., 2015), Sr^{2+} (Lorens, 1981; Tesoriero and Pankow, 1996), SO_4^{2-} (Busenberg and Plummer, 1985) and Ba^{2+} (Tesoriero and Pankow, 1996), and decrease incorporation of Mg^{2+} (Burton and Walter, 1991), Mn^{2+} , Co^{2+} and Cd^{2+} (Lorens, 1981). Foraminiferal calcite growth rates have been estimated previously by determining rates of chamber addition (Segev and Erez, 2006) and by measuring changes in alkalinity over time (Duguay and Taylor, 1978; Erez, 2003; ter Kuile and Erez, 1984). However, calcite precipitation by foraminifera is episodic rather than continuous, since they intermittently add chambers to their shell and forage for food (Tyszka et al., 2019). Therefore, quantifying calcite precipitation rates requires analysing fluxes of calcite addition during a single chamber formation event.

Precipitation rate during foraminiferal biomineralization is likely largely controlled by the flux of ions to the calcifying fluid (De Nooijer et al., 2014b; Erez, 1983; Toyofuku et al., 2017), which is secluded from seawater by a membrane (Angell, 1979; Bé et al., 1979; Nagai et al., 2018b). Also active removal and/or blocking of inhibitors of calcite precipitation such as Mg^{2+} and SO_4^{2-} may be part of the foraminiferal strategy to promote calcite precipitation (Zeebe and Sanyal, 2002). Both of these processes have a major impact on calcite precipitation rate and element incorporation. Recently, meta-stable pre-cursor phases were observed in foraminifera during calcification, suggesting crystal growth rates should be faster than classical monomer-addition (Jacob et al., 2017). High rates through a pathway involving an amorphous pre-cursor phase would greatly effect element incorporation (Blue et al., 2017; Bots et al., 2012; Littlewood et al., 2017). Constraining the rate of calcium carbonate precipitation is hence pivotal for understanding element partitioning in foraminiferal shell carbonate.

Here we determine calcification rates during the formation of single foraminiferal chamber walls by measuring the added shell wall thickness over time. We used pH probing to monitor the duration of the chamber formation event, and added Sr-labels at set time-intervals to quantify the thickness of the calcite layer formed during each event (Fig. 1). Visualization of the Sr-label and Na^+ , Mg^{2+} , and SO_4^{2-} , by Nanoscale Secondary Ion Mass Spectrometry (NanoSIMS) of cross-sections of the newly formed chambers

subsequently allowed calculating precipitation rates during chamber formation and comparison to the within-wall element signal (Fig. 1). The low-Mg calcite benthic foraminifer *Ammonia beccarii* (Fig. 1) is used as a model-species for many studies on foraminiferal biomineralization, foremost due to its relatively high resilience to varying environmental conditions (De Nooijer et al., 2009a; Nehrke et al., 2013; Toyofuku et al., 2017). Moreover, its elemental composition, and specifically its low Mg content, closely resembles that of planktic species used in climate reconstructions. Results are compared to inorganic calcite precipitation studies to identify whether precipitation rates and element incorporation are fully biologically controlled.

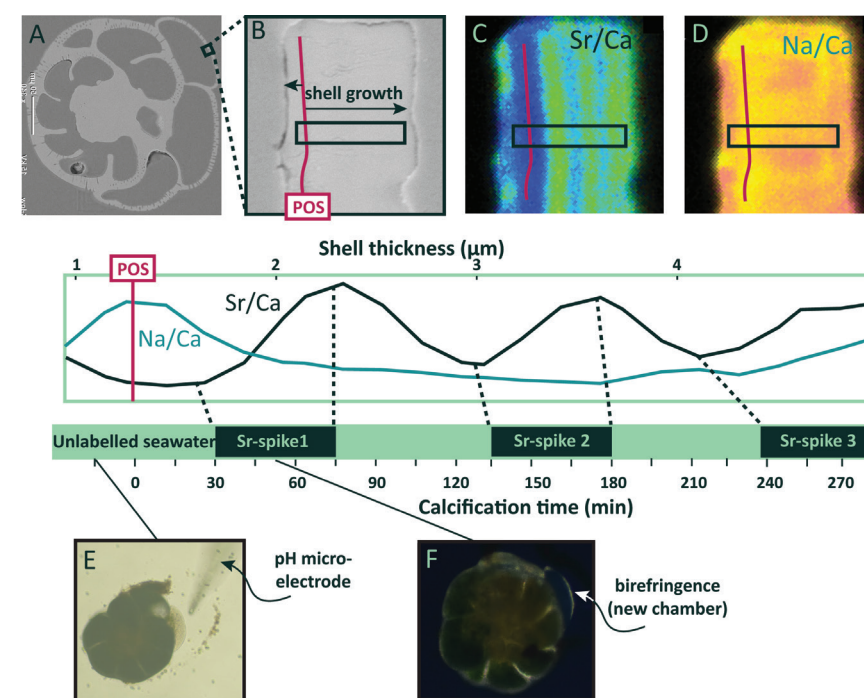


Figure 1. Spiking scheme and tie-points to lateral Na/Ca and Sr/Ca NanoSIMS profiles. The position of the POS and the spacing of the Sr spikes were used as time constraints to calculate calcification rates. The newly formed chambers (A; yellow square) were exposed by embedding and polishing the analyzed specimens. Close up of this chamber (B) revealed the position of the primary organic sheet (POS) and the thin inward and more extensive outward precipitated calcite layers. NanoSIMS mapping revealed the Sr-labels, from spiking of the medium (C) and the Na/Ca of the newly formed chamber (D). During chamber formation, the tip of the microelectrode was positioned as close to the newly formed chamber as possible (E). The onset of calcification was recognized as a thin layer of birefringence (F), gradually thickening as chamber formation commenced.

4.2 Materials and methods

4.2.1 Experimental set-up and Sr labelling

Living foraminifera were collected from brackish-water salt marsh sediments of Hiragata Bay, Natsushima-cho Yokosuka, Japan (35 19'21"N, 139 38'5"E), in spring of 2015. Surface (top ~5 mm) sediments were collected by palms into a plastic bucket and transported to the laboratory to serve as a stock from which individuals of the shallow benthic calcareous foraminifera *Ammonia beccarii* (sensu De Nooijer et al., 2009) were isolated. This study was conducted using a breeding stock (Lab stock name: Am-S) asexually reproduced from a single individual. Specimens of *Ammonia beccarii* were isolated and fed with fresh algae *Dunaliella salina*. After offspring, a large number of individuals (~300) were distributed over ~30 Petri dishes and were cultured until reaching size of ~10 chambers. Every evening, debris in the Petri dishes was removed, the 0.2 µm filtered natural seawater was replaced and fresh *D. salina* was supplied. Every morning, specimens were monitored for signs of upcoming chamber formation events, which can be recognized by pseudopodia being subtracted and by the placement of protective cyst (consisting of algae-debris) in a circle around the chamber-to-be-formed and eventually the extrusion of cytoplasm and formation of the POS. When there were early signs of an upcoming chamber formation event, the pH-microelectrode and reference electrode were set-up in the vicinity of the new chamber. During chamber formation, commonly lasting between 4 and 7 hours, images were taken with double polarized light microscopy, to monitor the onset of calcite precipitation and on-going chamber thickening. After the POS was formed, a decrease in pH (~0.2) indicated the start of chamber formation, which was confirmed by the appearance of a thin birefringent layer at the POS around 10 minutes later (appendix A). The pH in the foraminiferal microenvironment was measured near the outer membrane of the site of calcification (maximum of ~10 µm away, see 2.2 and appendix A), to indicate the start of calcite formation and to monitor the relationship between pH and calcification rate. Therefore, we used pH-microsensors (Unisense A/S Denmark) with a tip-size of 10-25 µm, which were calibrated prior to the measurement, a reference electrode and a robotic arm, following previously described protocols (Glas et al., 2012).

A Sr-enriched stock solution was made by adding 2.215 mg of SrCl₂ salt (197-04205, FUJIFILM Wako pure chemical corporation) to 200 grams of distilled water (milli-Q). Two consecutive 'spikes' of 150 µL of the Sr-enriched stock solution were added to the Petri dish, containing 15 ml of 0.2 µm filtered natural seawater, at set intervals (30 minutes in between Sr-spike 1 and Sr-spike 2, Fig. 1 and Appendix A). Together, the two spikes increased the Sr concentration 20 times, a concentration resulting in detectable

shell concentrations, without hampering foraminiferal growth. 60 minutes after addition of the second spike, the entire culture medium of the Petri dish was replaced with 'un-spiked' 0.2 µm filtered natural seawater and left for 60 minutes. This spiking procedure was repeated until the specimen extruded its pseudopodia again and started to move, and chamber formation was completed. Hereafter, the specimen was removed from the Petri dish and shortly placed into double de-ionized water for gentle rinsing to terminate calcification. The specimen was then treated with a buffered 1% H₂O₂ solution and heated to 90 °C to remove cytoplasm. Specimens were subsequently embedded in epoxy resin (Araldite 2020) and cured for 24 hours at 60°C.

Specimens were polished using silicon carbide wet grinding papers with decreasing coarseness (HERMES, WS Flex 18C, 230 mm, P 800 and 219 ATM, SIC wet grinding paper, grain 4000). This resulted in a cross-sectioned sample with the newly formed chamber wall exposed perpendicular to the shell walls, as evaluated by light microscopy. Exposed cross sections were subsequently fine-polished using agglomerated alpha alumina powder (Struers AP-A powder, grain size 0.3 µm) and SiO₂ powder (Logitech SF1 Polishing Suspension, grain size 0.035 µm). Finally, the polished samples were ultrasonically cleaned with ethanol, and coated with a 20 nm gold layer using a sputter coater (JEOL JFC-2300HR high resolution fine coater and JEOL FC-TM20 thickness controller).

4.2.2 SEM and NanoSIMS imaging and data-analysis

Imaging analyses by scanning electron microscopy (SEM) and nanometer-scale secondary ion mass spectrometry (NanoSIMS) were performed at Utrecht University. SEM imaging was done with a JEOL Neoscope II JCM-6000 instrument using a backscattered electron detector. SEM images were taken to identify areas suitable for NanoSIMS imaging and to locate the primary organic sheet (POS) and subsequent organic linings. NanoSIMS analysis was performed with the CAMECA NanoSIMS 50L instrument. Using an element standard (SPI Supplies, 02757-AB 59 Metals & Minerals Standard), magnetic field and exact positions of the electron multiplier detectors were adjusted to enable detection of either positive (23Na⁺, 24Mg⁺, 42Ca⁺, 44Ca⁺, 88Sr⁺) or negative (16O⁻, 32S⁻) secondary ions. The positive and negative secondary ions were detected using an 8 keV primary O⁻ and Cs⁺ ion source, respectively. First, areas of interest were pre-sputtered until secondary ion counts stabilized. The same pre-sputtering protocol was used on all samples to ensure comparability between different fields of view (FOV) measured. Subsequently, ion count images were acquired by rastering the primary beam over the sample surface (areas between 8×8 and 40×40 µm in size) using the following diaphragm and slit settings: D0-2, D1-3, ES-2, AS-0 and EnS-0 for the O⁻ beam, and D0-0, D1-3,

ES-2, AS-2 and EnS-1 for the Cs⁺ beam. These settings yielded a primary O⁻-beam at the sample surface of ~2 pA with a nominal size of 300 nm, resulting in a spatial resolution of 355 nm, and a primary Cs⁺ beam of ~0.5 pA with a nominal size of ~100 nm, as defined and tested by CAMECA for the NanoSIMS instrument used.

The settings allowed high transmission at sufficient mass resolution to separate isobaric interferences with the exception of the $^{44}\text{Ca}^{2+}$ dimer on mass 88. The contribution of $^{44}\text{Ca}_2^+$ to ^{88}Sr is expected to be less than 1% (estimated from $^{44}\text{Ca}^+$ ion counts following assumptions as in Gabitov et al. (2013), modified for isotopic abundance) and was not corrected for since images are not used for quantitative purposes. Secondary ions were detected with a dwell time of 2 ms/pixel (positive ions) and 0.8 ms/pixel (negative ions). To increase the overall signal the same FOV was imaged multiple times (400–1000), and the resulting ion count images were aligned and accumulated.

Data processing was done using the freeware program Look@NanoSIMS (Polerecky et al., 2012) as well as additional custom-made routines in Matlab. Lateral profiles were drawn from the NanoSIMS images to allow reconstruction of the precipitation rates based on the Sr label (Fig 1). Profiles for El/Ca (for positive ions) and El/O (for negative ions) ratios along lines were oriented perpendicularly to the labeled Sr-bands. When defining the lines, pixels close to edges, cracks or pores within the shells were avoided. Lateral profiles were set to a width of 10 pixels to improve signal-to-noise ratio. For negative ions, El normalized to O as the Ca:O ratio is fixed in the calcite, plus the detected O ions are homogeneous through the calcite shell, and thus a good proxy for the Ca²⁺-concentration, which could not be measured with the Cs⁺ beam)

4.2.3 Calculation of calcification rates

Calcification rates were determined using tie-points between the spatial position of peaks and troughs in the Sr-label profiles, derived from NanoSIMS imaging, and the timing of addition and removal of the Sr-spike in the culture medium (Fig. 1). This method assumes laminar addition of calcite in the direction perpendicular to the POS, which is confirmed by the relatively straight and parallel Sr bands observed in the NanoSIMS images (Fig. 2). We hereby only take into account the growth towards the outside of the shell, as the position of the Sr-peak on the inside of the shell, and thus its distance to the POS, could not be accurately determined in all specimens. The position of onset of the Sr-increase in the Sr profile (the Sr-trough or inflection point) is tied to the timing of addition of the first Sr-spike, as the Sr in the calcite starts to increase when Sr is increased in the culture medium. The position of the Sr-peak, before the Sr signal starts to decrease again, is connected to time where the Sr-spike in the culture medium is removed. The

start-time of calcification was indicated by the pH-drop adjacent to the new chamber, as measured with the pH microelectrode for 3 out of 4 specimens, and / or by the appearance of a thin birefringent layer around the POS (Fig 1). The start position of calcification (the POS) is based on the position of the Na and K peaks in the lateral profiles drawn from the NanoSIMS images, as these elements have been shown to be elevated at the POS (Fig. 1, Branson et al., 2016; Geerken et al., 2019), which is further confirmed by the position of the POS in the SEM images (Fig 1., Appendix A).

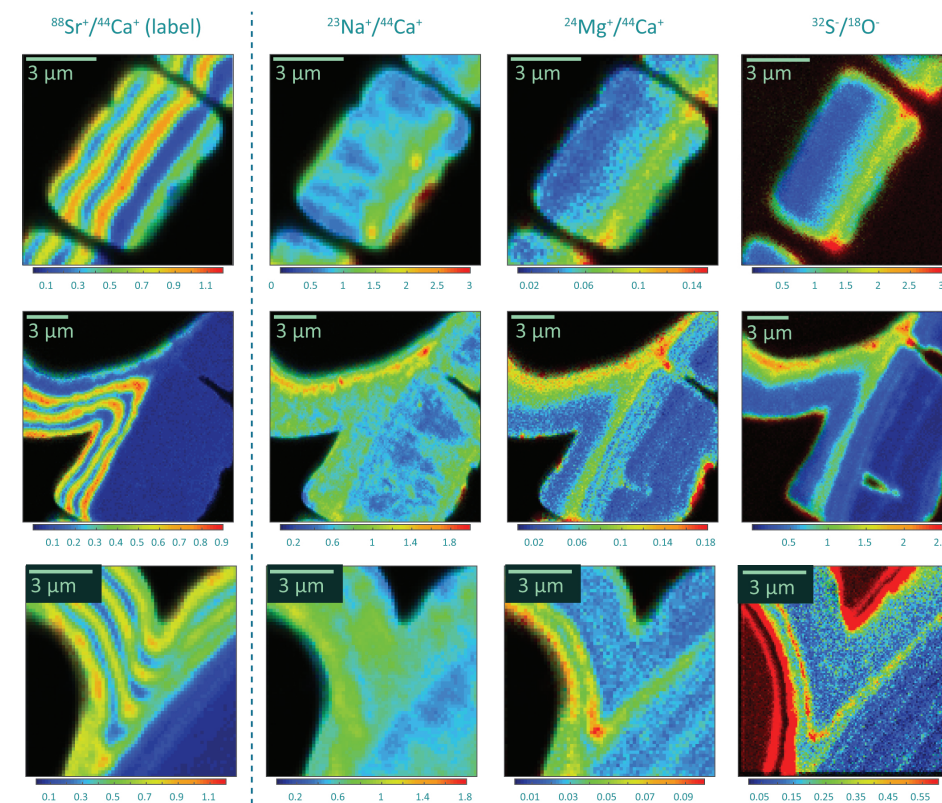


Figure 2. NanoSIMS images showing the Sr labelling (left) and the natural variability in Na/Ca, Mg/Ca (middle panels) and S/O (right) within walls of three specimens of *Ammonia beccarii*.

4.3 Results and discussion

4.3.1 Calculating foraminiferal precipitation rates

All NanoSIMS images of the cross-sectioned newly formed chambers consistently show Sr-labelled bands parallel to the inner and outer surface of the shell wall (Fig. 2). Within the shell wall, ~3–4 Sr-labels are visible, placed at regular intervals, reflecting the timing

of the Sr-spikes in the culture medium (Fig. 1). We used Sr as a label since Sr is distributed relatively homogeneously in foraminiferal chamber walls (Geerken et al., 2019). The 'banded' pattern of the label (Fig. 2) is in accordance with laminar calcite addition onto the 'primary organic sheet' (POS), an organic template that is secreted prior to calcification (Banner et al., 1973; Hemleben et al., 1977; Spero, 1988). Calcification first occurs bi-directionally, with the first Sr-label being deposited at both the inside and outside of the POS (Fig. 1). Consecutive labels are observed exclusively on the outer calcite layer, showing that calcification occurs uni-directionally (i.e. towards the outer surface of the chamber wall) with on-going chamber formation.

The POS is slightly undulating (Spero, 1988), which may somewhat increase the surface area upon which the first calcite nucleates. However, the potential effect of an undulating organic matrix and concomitant undulating calcite growth front is likely minor, as eventually the integrated net growth is unidirectional, from the POS towards the outside, resulting in a smooth shell surface. The incorporation of time-controlled Sr^{2+} -spikes in this study confirms that the calcite growth front runs parallel to the POS (Fig. 2). Unidirectional growth, rather than calcite growth from multiple directions (e.g. from pore walls and inwards from the outer organic lining), is also apparent from the gradual and even increase in birefringence (Fig. 1). This unidirectional growth is furthermore confirmed by focused ion-beam observations of the site of calcification (SOC) of specimens captured during different stages of chamber formation, which shows that calcite is deposited on the POS at the onset of chamber formation and extends towards the outer membrane in later stages of calcification (Nagai et al., 2018b).

Timing of the first Sr^{2+} -spike relative to the onset of calcification as identified from pH monitoring varied between specimens. When the Sr-label was added at the start of calcification, high Sr^{2+} levels are observed directly around the POS (appendix A), whereas later addition of the Sr^{2+} -spike (e.g. 30 minutes after the start of chamber formation) resulted in the Sr^{2+} -label being located somewhat away from the POS (Fig. 1). This implies that the Sr-spike is taken up instantly from the culture medium, or at least that the Sr^{2+} -label starts to be incorporated into the calcite without a major time lag. NanoSIMS profiles of the Sr^{2+} -label, however, do not reflect the instantaneous increase in Sr^{2+} concentration (Fig. 1). This may imply that the Sr^{2+} concentration in the medium and in the calcifying space is gradually (i.e. within ~30 minutes) equilibrating. Alternatively, it may be attributed to the NanoSIMS' O-beam resolution of ~300 nm (Geerken et al., 2019), which would not allow to reproduce a stepwise increase in Sr/Ca in the calcite. However, both equilibration and resolution would result in the observed sinus-shape of

the Sr^{2+} -label, which does not change the spacing between consecutive Sr^{2+} -labels and hence does not affect the calculated rates.

Calculating precipitation rates from calcite addition for foraminifera is not trivial due to the curved surface area of the shell wall and because calcification is not necessarily constant over time. These complicating factors are circumvented by assessing precipitation rates perpendicular to the shell surface, using not only the exact start and end time of calcification, but also the internal time markers provided by the Sr-labelling. Based on laminar calcite addition, shell-wall growth of the newly formed chambers over time is relatively constant between specimens, at $\sim 0.49 \pm 0.05 \mu\text{m/h}$ (mean $\pm$ 1 SD of mean rates per specimen), based on Sr-profiles of 13 NanoSIMS images from 4 specimens (Fig. 3). Converting these calcification rates to fluxes, to allow comparison to inorganic precipitation rates, results in relatively high precipitation rates of $\sim 22 \pm 2 \text{ nmol/cm}^2/\text{min}$. This conversion uses a calcite specific density of 2.711 g/cm^3 and the molar weight of calcite (see Appendix B for calculation). During the precipitation of a new chamber, the previously built chambers are also covered with a new layer of calcite (Nehrke et al., 2013). New calcite lamella for some individuals extend over the entire specimen, but and in others only over the neighbouring chambers (Nehrke et al., 2013). Sr-label bands within this additional layer become thinner and more closely spaced (Appendix C). This may imply slower precipitation rates away from the newly added chamber, however the spacing between Sr^{2+} labels is too close to allow calculating precipitation rates further away from the added chamber within our resolution.

Previously reported rates ($0.6\text{--}80 \text{ nmol/cm}^2/\text{min}$ for planktic and $\sim 1.33\text{--}430 \text{ nmol/cm}^2/\text{min}$ for benthic species) were based on alkalinity measurements and assumed continuous calcification (Anderson and Faber, 1984; Duguay and Taylor, 1978; Erez, 1983; ter Kuile and Erez, 1984). Duration of chamber addition events has been previously determined for *A. beccarii* using pH-microelectrodes, recording a pH drop during chamber addition (Glas et al., 2012), comparable to the decrease in pH reported here (Appendix A). However, this approach assumed a constant thickness of $3 \mu\text{m}$ for the new chamber. Converting their rates to $\text{nmol/cm}^2/\text{min}$, using a shell thickness of $3 \mu\text{m}$ and the range of observed calcification times, results in values of $\sim 33 \text{ nmol/h}$ ranging between 19 and 135 nmol/h due to differences in measured duration of calcification versus a constant assumed increase in shell thickness. Our approach incorporates both chamber thickness and time control during a chamber formation event; rates are consequently more accurate and precise compared to previous estimates.

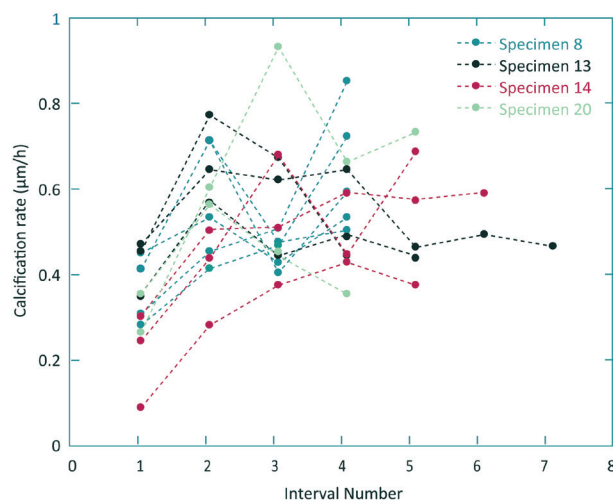


Figure 3. Calculated calcification rates ($\mu\text{m/h}$) for time-intervals within the shell walls of *A. beccarii*, where interval '1' is the interval next to the POS and only the layer precipitated towards the outside is taken into account. Number of images =13, number of specimens = 4. The rate of interval 1 is significantly lower than later intervals (for percentage change between intervals, averaged per specimen $p\text{-value}=0$, $F\text{-test}=5.9 \times 10^{16}$, MATLAB 'multcompare' test based on ANOVA).

4.3.2 Intra-shell variability: precipitation rates and element banding

Calcification rate is significantly lower during the earliest phase of shell formation, compared to the rest of the shell wall (Fig. 3). This may be explained by the presence of calcite growth inhibitors like Mg^{2+} and SO_4^{2-} (Paquette and Reeder, 1990; Paquette and Reeder, 1995), resulting from seawater enclosure at the onset of calcification through 'self-vacuolization'¹⁸. At this stage, relatively high concentrations of these ions inhibit calcification by distortion of the calcite crystal surface morphology, due the large hydration spheres of Mg^{2+} compared to Ca^{2+} , and the subsequent increase in the proportion of crystal faces with a lower affinity for Ca^{2+} (Davis et al., 2004; Zhang and Dawe, 2000). Besides Sr^{2+} , also Ca^{2+} reaches the site of calcification (SOC) instantaneously during chamber formation (Appendix D), in line with trans-membrane Ca^{2+} -transport (Nehrke et al., 2013), which is suggested to be driven by proton-pumping and CO_2 diffusion into the SOC (Toyofuku et al., 2017). Therefore, lower precipitation rates at the onset of chamber formation may also be explained by a lower flux of Ca^{2+} and CO_3^{2-} into the SOC, since an efficient carbon capture mechanism relies on a strong inward-outward pH gradient, which may take some time to fully establish (see Glas et al., 2012; Toyofuku et al., 2017 and Appendix A).

The presence of seawater-like ion concentrations at the SOC at the onset of chamber formation may explain enhanced trace element incorporation at this stage, resulting in

so-called 'element banding' around the POS in this species (Fig. 2; Geerken et al., 2019; Geerken et al., 2018). Element banding towards the end of chamber wall formation might be explained by a combination of Rayleigh fractionation and a decrease in Ca^{2+} -fluxes towards the end of chamber formation (Elderfield et al., 1996; Marchitto et al., 2018). Involvement of seawater furthermore explains why element banding is not limited to specific elements, but observed for a wide array of elements (Geerken et al., 2019; Geerken et al., 2018; Kunioka et al., 2006; Van Dijk et al., 2017a). Element banding may therefore be particularly apparent for elements with a low partitioning in foraminiferal calcite, related to a strong biological control that limits their transport into the SOC, such as Mg^{2+} and possibly SO_4^{2-} , while Ca^{2+} is transported inwards. Hence, the first layer of calcite precipitated on the POS is still impure because it contains all sort of ions from seawater, while subsequent calcite is purer because of the stronger biological control (Fig. 2), which is reflected in both precipitation rates being higher and less trace elements incorporated at this stage of calcification (Fig. 3). At the end of chamber formation, this biological control may decrease again, also indicated by an increase in pH (Appendix A), explaining increased trace element concentrations sometimes also observed towards to outside of the chamber (Geerken et al., 2019; Jonkers et al., 2016). The concentration of calcite growth inhibitors in the SOC hence appears to be actively reduced to enhance calcification rates with ongoing calcification (Figs. 2 and 3). This has been hypothesized to be achieved either by selective ion removal (Bentov and Erez, 2006; Erez, 2003) or by limiting their access to the SOC during calcification (Zeebe and Sanyal, 2002). The mechanism by which foraminifera control ion concentrations in the SOC may differ between species, but is crucial in explaining the element composition of their calcite and their dependency on environmental parameters. Further studies may therefore test which other elements, besides Sr and Ca, reach the SOC directly during calcification and whether the inward transport of ions differs between species.

4.3.3 Comparing of foraminiferal and inorganic precipitation rates

Inorganic precipitation rates depend on solution chemistry whereby high rates are obtained by 1) absence of calcification inhibitors such as Mg^{2+} and SO_4^{2-} , 2) high CaCO_3 saturation states (Fig. 4), and 3) a $[\text{Ca}^{2+}:\text{CO}_3^{2-}]$ stoichiometry close to one (Nehrke et al., 2007). The relatively high foraminiferal precipitation rates observed here may hence be obtained by a combination of these factors. Most inorganic calcite precipitation experiments using seawater-like solutions show lower rates (0.06-8.5 $\text{nmol/cm}^2/\text{min}$) (Anderson and Faber, 1984; Duguay and Taylor, 1978; Erez, 1983; ter Kuile and Erez, 1984) than those observed here for foraminifera (Fig. 4). Foraminiferal precipitation rates are more in line with inorganic calcite precipitation studies using Mg-free solutions, with rates ranging from 0.16 to 90 $\text{nmol/cm}^2/\text{min}$ (Fig 4; Nehrke et al., 2007; Tang et al., 2008;

Tesoriero and Pankow, 1996). This suggests that foraminifera may precipitate their shell from a solution comparable in saturation state and stoichiometry to seawater, but with considerably lower Mg concentrations.

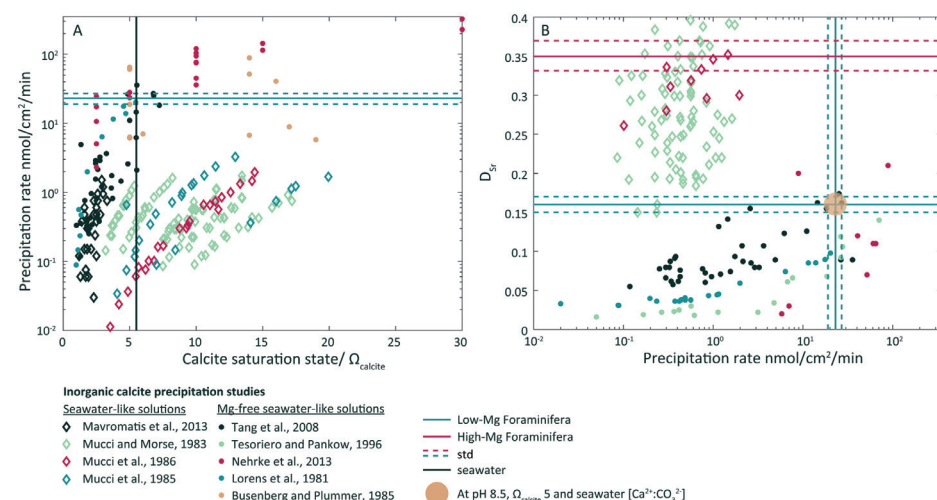


Figure 4. Panel A. Precipitation rates for inorganic precipitation studies, with open diamonds showing results for experiments with seawater-like solutions containing Mg and closed circles representing Mg-free seawater-like solutions, against solution omega, with seawater omega indicated by a red dashed line for comparison. Panel B. Sr partitioning (D_{Sr}) of inorganic precipitation studies against precipitation rate, with open diamonds showing results from experiments using seawater-like solutions containing Mg and closed circles Mg-free seawater-like solutions. Foraminiferal D_{Sr} (Van Dijk et al., 2017b) and precipitation rates obtained in this study are indicated with blue lines for low Mg species. Foraminiferal D_{Sr} for high Mg species (Van Dijk et al., 2017b) are indicated with green lines, precipitation rates are unknown.

The incorporation of minor and trace elements into calcite is often quantified by a partitioning coefficient, which is expressed as $D_{\text{el}} = \text{ElCa}_{\text{calcite}} / \text{ElCa}_{\text{seawater}}$. Incorporation of Sr into calcite from seawater is directly related to precipitation rate, which is partly explained by an increase in lattice defects and subsequent substitution of Sr- for Ca-ions (Lorens, 1981). Close to equilibrium precipitation rates ($<0.05 \text{ nmol/cm}^2/\text{min}$) the effect of rate on D_{Sr} is not detectable (Carpenter and Lohmann, 1992), yet at high, far-from-equilibrium rates ($0.05\text{--}100 \text{ nmol/cm}^2/\text{min}$) D_{Sr} is positively correlated to precipitation rate (Lorens, 1981; Nehrke et al., 2007; Tang et al., 2008; Tesoriero and Pankow, 1996). The relationship between precipitation rate and Sr incorporation from inorganic experiments has been used before to predict foraminiferal precipitation rates (Böhm et al., 2012; Lorens, 1981), with a minimum rate of 17 and best estimate at $26 \text{ nmol/cm}^2/\text{min}$, which fit our observation of $22 \text{ nmol/cm}^2/\text{min}$ remarkably well. Low-Mg foraminifera

and inorganic calcite precipitated from Mg-free seawater-like solutions hence show a similar D_{Sr} as known from literature (Geerken et al., 2018), at similar precipitation rates (Fig. 4). This confirms the hypothesis that foraminifera precipitate their calcite at non-equilibrium rates and that kinetic effects explain the relatively high incorporation of Sr (Böhm et al., 2012; Kısakürek et al., 2008).

The slope between precipitation rate and D_{Sr} is also influenced by pH, whereby a higher pH will increase D_{Sr} (Tang et al., 2008). The best fit between foraminiferal as well as inorganic D_{Sr} with precipitation rate is obtained for a relatively high pH of ~ 8.5 (Tang et al., 2008). This is in agreement with observations of an elevated intra-cellular pH ($\sim 8.5\text{--}9$) in foraminifera (Bentov et al., 2009; De Nooijer et al., 2009b). Our results also suggest that the Sr/Ca at the SOC may be similar to that of the surrounding seawater, which is confirmed by Ca and Sr isotope fractionation (Böhm et al., 2012). Instantaneous uptake of the Sr-label and subsequent incorporation in the shell wall, at least on the resolution studied here, suggests open equilibration of Sr between the calcifying fluid and seawater.

4.3.4 Synthesis: implications for foraminiferal biomineralization and proxies

The precipitation rates obtained here provide a crucial constraint for explaining foraminiferal shell geochemistry (Fig. 4). Other, yet poorly quantified, factors that have been suggested to control foraminiferal element incorporation are CaCO_3 phase transformations (Jacob et al., 2017), involvement of organic templates and molecules (Branson et al., 2016) and biological control on the element chemistry of the site of calcification (De Nooijer et al., 2014b; Elderfield et al., 1996; Erez, 2003). Since Sr^{2+} and Ca^{2+} are taken up into the SOC instantaneously during calcification, possibly through accidental transport by trans-membrane Ca-pumps, Sr/Ca in the SOC is likely comparable to Sr/Ca of seawater. Our precipitation rate results enable comparison between element partitioning in abiotic and foraminiferal calcite and shows that foraminiferal D_{Sr} can be explained by high precipitation rates and reduced Mg^{2+} concentration in the SOC, relative to precipitation from seawater (Fig. 4). This hence allows disentangling the kinetic and biological components affecting foraminiferal element incorporation and provides new insights into the conditions prevailing at the SOC during calcification. Extending the comparison to abiotic precipitation studies implies that the relatively high foraminiferal CaCO_3 precipitation rates can be obtained from a SOC solution which is low in Mg, with a seawater-like saturation state (Fig. 4), at a relatively high pH of 8-9 (Tang et al., 2008) compared to seawater.

To maintain a seawater-like saturation state in the SOC, which is kept secluded from seawater by a membrane (Nagai et al., 2018b), Ca^{2+} and CO_3^{2-} need to be continuously

supplied to the SOC. As Ca^{2+} is taken up directly during calcification (Appendix D), Ca^{2+} pumps or channels may well be involved in foraminiferal biomineralization (Marchitto et al., 2018; Toyofuku et al., 2017), similar to those responsible for calcification in scleractinian corals (Gagnon et al., 2007). Their involvement would also explain our observation of Sr reaching the SOC directly, since Ca^{2+} pumps are suggested to be permeable for Sr^{2+} (Gagnon et al., 2007; Marchitto et al., 2018). Ca-pumping is in line with a recently proposed model for foraminiferal calcification (Toyofuku et al., 2017), which proposes that during calcification, calcium ions (pumped in) are exchanged for protons (going out), resulting in a strong pH gradient that drives an inward CO_2 diffusion. If this DIC supply to the SOC matches the inflow of calcium, as suggested by a relatively constant proton flux over time (Toyofuku et al., 2017), the saturation state in the SOC remains relatively high and remains relatively close to 1, comparable to seawater levels. Both these factors would promote the relatively high and constant foraminiferal precipitation rate as observed here, once the proton-flux is constant. In contrast, the initial (and possibly the last) calcite layer precipitates at a slower rate (Fig. 3), from a less biologically controlled, more seawater-like solution, while the proton- Ca^{2+} flux is not optimized.

For elements that are excluded from the SOC during calcification such as Mg^{2+} , foraminiferal element partitioning cannot be directly compared to that from abiotic studies, because El/Ca (Element/ Ca^{2+}) ratios in the SOC are altered compared to seawater. Limited transport of some elements by transmembrane Ca^{2+} -pumps could be due to differences in charge (e.g. for Na) and ionic radius (e.g. for (hydrated) Mg). The impact of precipitation rate on element partitioning depends on the element/Ca ratio. When the impact of precipitation rate would be fully resolved for other elements, the El/Ca ratio of the SOC can be inferred. Our study hence highlights the need for quantification of inorganic element partition coefficients in experiments at high non-equilibrium rates, possibly including transient pre-cursor phases and mimicking conditions in the SOC as much as possible.

Not all foraminiferal species precipitate calcite with a low-Mg content like *A. beccarii* studied here. Species precipitating calcite with a higher Mg content may hence have a SOC more open to seawater which raises Mg concentrations, in line with observations of seawater vacuolization in intermediate-Mg foraminiferal species (Bentov et al., 2009). This may also explain why higher-Mg species also show higher concentrations of most other ions, such as Na^+ , SO_4^{2-} , and Sr^{2+} (Van Dijk et al., 2017b) and more apparent element banding (Geerken et al., 2019). The positive correlation between Sr and Mg between foraminiferal species has also been explained by a lattice effect related to Mg-

uptake, whereby Mg ions distort the calcite lattice allowing more Sr to be incorporated (Mucci and Morse, 1983), as is observed in abiotic calcite precipitation studies (Fig. 4). Foraminifera with a relatively high Mg^{2+} content may hence precipitate at lower rates due to the inhibitory effect of Mg^{2+} , while incorporating more Sr^{2+} due to the lattice strain effect. This may hence resolve the apparent contradiction of higher D_{Sr} found for specimens of lower precipitation rates (Erez, 2003).

The effect of temperature on Mg incorporation in foraminifera is bigger than can be explained by thermodynamic effects only. The high precipitation rates in foraminifera found here imply that the effect of temperature on Mg partitioning can only be compared to Mg partitioning under kinetic rather than thermodynamic control. Additionally, the temperature effect on foraminiferal Mg^{2+} incorporation may be affected by a temperature dependence of the biological, organismal control on Mg^{2+} (Branson et al., 2013).

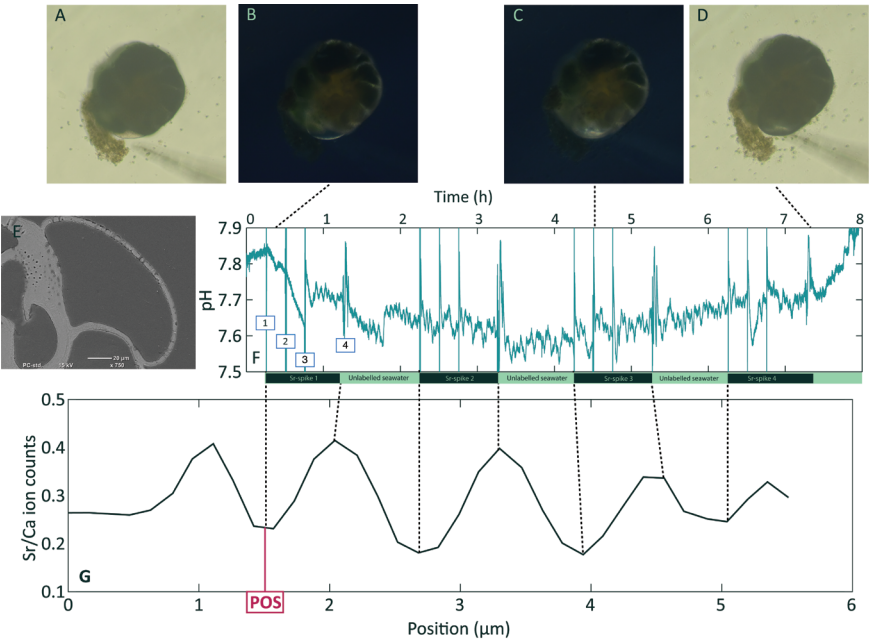
4.4 Conclusions

Here we show that calcite precipitation rates in the benthic foraminifer *Ammonia beccarii* are relatively high compared to rates obtained for inorganic calcites precipitated from seawater-like solutions. Furthermore, rates are remarkably constant between specimens, with an average of $\sim 22 \pm 2$ nmol/cm²/min. Comparison to inorganically precipitated calcite at the same rate shows that both rate and Sr partitioning in foraminiferal calcite can be explained by low Mg concentrations in the calcifying fluid, a seawater-like saturation state and a pH of ~ 9 . Within chamber walls, the first layer of calcite is formed at a significantly lower rate, coinciding with bands of high Mg/Ca and Na/Ca around the POS. These results provide an important constraint to test the applicability of element partition coefficients derived from inorganic precipitation studies, which may explain foraminiferal calcite element composition and dependence on environmental variables.

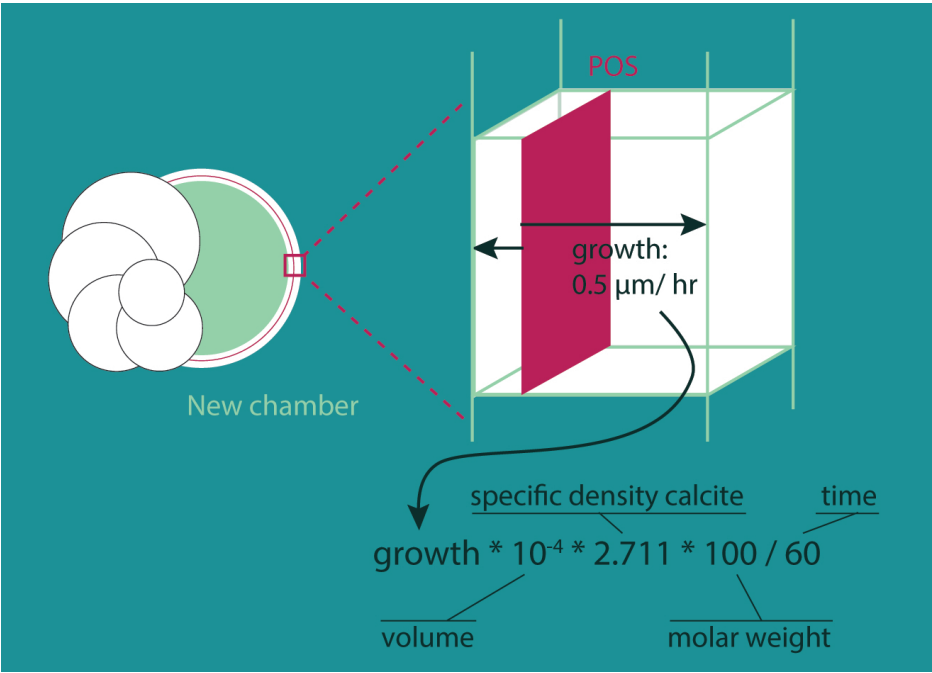
Acknowledgements

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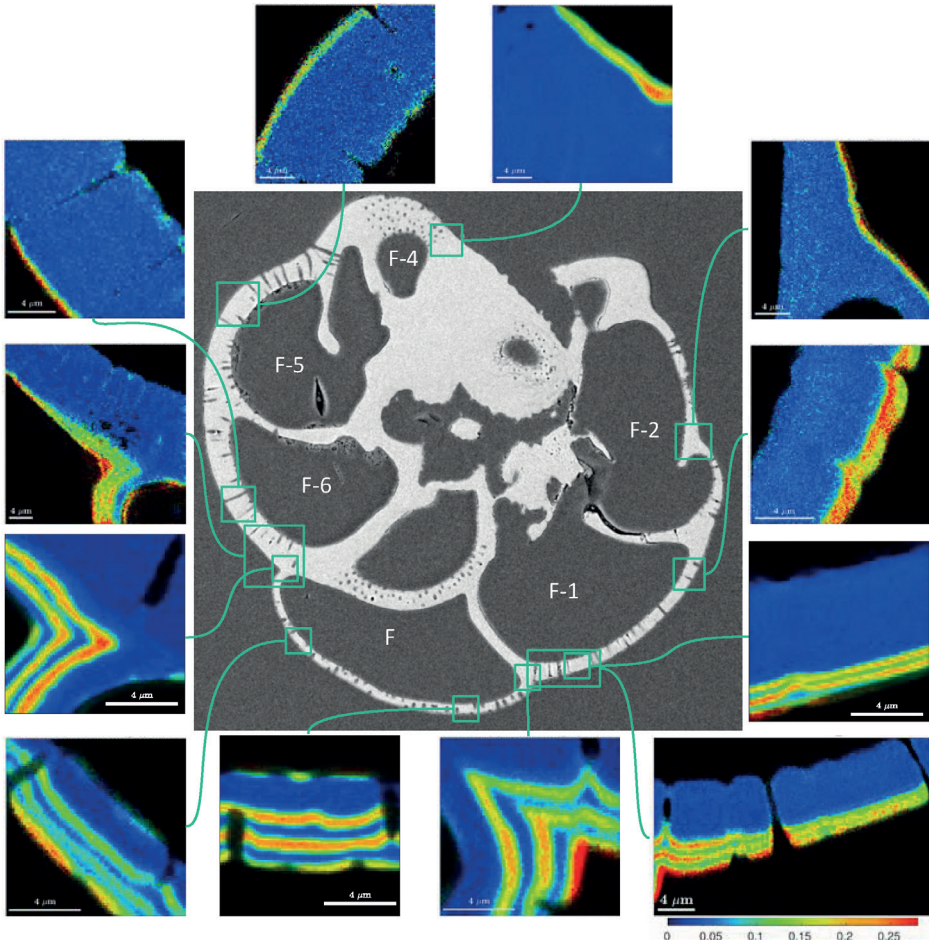
Appendix



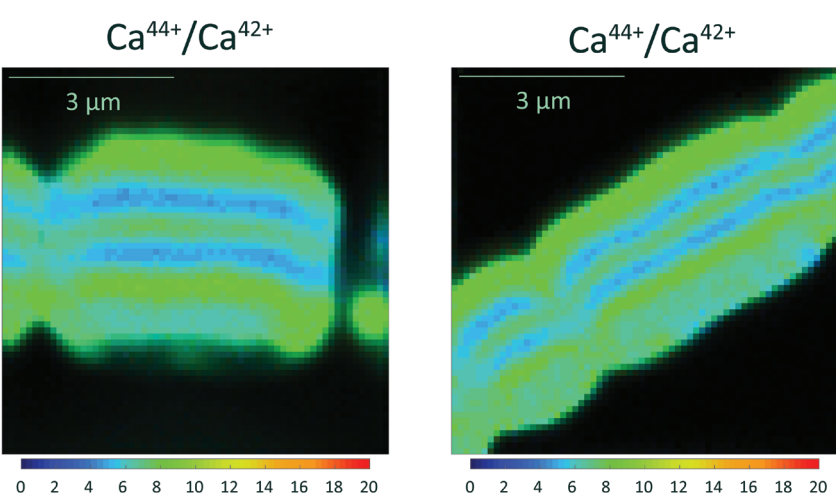
Appendix A. Compilation of spatial and temporal observations for a specimen for which the Sr-spike was added at the start of calcification. Panel A. *A. beccarii* specimen extruding the ‘cytoplasmic bulge’ before the POS is formed, and the position of the pH-probe, providing space to the extruding cytoplasm. Panel B. The onset of calcification is marked by the appearance of a thin birefringent layer at the surface of the POS, visible using double polarized filters. Panel C. Continuation of calcification is followed by brighter birefringence covering the entire new chamber. Panel D. Position of pH-microprobe during a later stage of calcification. Panel E. SEM image of a cross-section of the newest chamber. Panel F. pH-profile, showing the decreasing in pH at the onset of calcification, which decreases during the first ~4 hours of the calcification event, and starts to increase again during the last 3 hours. The pH profile is briefly disturbed by addition and removal of the spikes. Numbers indicate 1: first Sr-spike of 20 μL, 2: second Sr-spike of 20 μL, 3: and a Ba-spike, that could not be detected by NanoSIMS and 3: replacing the Sr-spiked medium with normal seawater Panel G. Sr-profile derived from NanoSIMS image, whereby tie-points between the position of Sr-peaks and troughs and time-intervals (addition and removal of the Sr-spike) are indicated with dashed lines.



Appendix B. The newly formed chamber (left) consists of a relatively thin layer of calcite precipitated at the inside and a thicker layer formed at the outside, separated by the primary organic sheet (POS; right image). The obtained increase in thickness (here simplified for the entire shell wall) is converted to a precipitation rate by assuming a completely filled volume (*10⁻⁴), a specific calcite density of 2.711 g/cm³, a CaCO₃ molar weight of 100 g/mol and converted to minutes by dividing by 60.



Appendix C. Compilation showing SEM image of the polished cross-section of an *A. beccarii* specimen embedded in resin, location of NanoSIMS images indicated with numbered red squares) and NanoSIMS Sr-images of the penultimate chamber (1), ultimate chamber (2) and corner of ultimate chamber and previous whorl (3). The Sr-labels are observed in the penultimate chamber, however the spacing of the bands is approximately halved compared to that in the ultimate (0) chamber. In the corner image, Sr-labels still show a similar spacing and width on both chambers.



Appendix D. NanoSIMS maps showing instant $^{44}Ca^{+}$ uptake during chamber formation, similar to uptake of the Sr-label. $^{44}Ca^{+}$ was enriched in the culture medium by addition of $^{44}CaCl_2$ (99% ^{44}Ca , batch no. 210501 (see Nehrke et al., 2013, for details) during set time-intervals and replaced with normal seawater in between these intervals, similar to our Sr-spiking method. A couple of calcifying specimens were spiked with $^{44}Ca^{+}$ and show a similar precipitation rates (22 nmol/cm²/min) as specimens labelled with Sr^{2+} , but are not included in the calculation for the precipitation rates presented here.



Chapter 5

Nile river discharge and temperature in the Eastern Mediterranean reflected in foraminiferal stable isotope and trace metal incorporation

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5.1 Introduction

The hydrological cycle has a pronounced impact on sea surface salinity, especially in front of large river systems. In restricted basins, such as the Mediterranean Sea, changes in fresh water inputs impact seawater circulation as it affect stratification and hence water column overturning (e.g. Rohling et al., 2015). Salinity is hence a crucial parameter to reconstruct past changes in the hydrological cycle and ocean circulation. Determining paleo-salinities depends on proxies, i.e., measurable chemical and/or biological preserved signals in the geological record that can be related to environmental parameters of interest. Currently, most reconstructions of salinity are based on combining proxies for paleo-temperature (e.g., Mg/Ca or $U^{K'}_{37}$) with the $\delta^{18}O$ measured on foraminiferal shells, to resolve for past seawater- $\delta^{18}O$ (Elderfield and Ganssen, 2000; Kasper et al., 2014). Subsequently, reconstructed seawater- $\delta^{18}O$ is used to derive salinity, after correcting for changes in ice volume and assuming that the relationship between salinity and seawater- $\delta^{18}O$ remains constant over space and time (Rohling and Bigg, 1998). However, in evaporative basins, such as the Mediterranean Sea, both river input and the precipitation-evaporation balance affect the seawater- $\delta^{18}O$ -salinity relationship (Pierre, 1999). This results in basin specific seawater- $\delta^{18}O$ -salinity relationships, which complicates down-core salinity reconstructions. This makes such basins excellent targets for applying non-traditional proxies that are more directly recording salinity.

The eastern Mediterranean Sea is a restricted basin, currently characterized by high salinity waters, due to the net evaporation balance within the basin. On geological time scales salinity varied significantly related to changes in this precipitation-evaporation balance. Salinity in the Eastern Mediterranean Sea varied over glacial-interglacial cycles due to the waxing and waning of ice-sheets, and its impact on sea level and associated excess evaporation (e.g. Grant et al., 2016; Grant et al., 2012) and more importantly on a precession scale due to changes in Nile discharge. During precession minima, intervals of increased summer monsoon precipitation resulted in wetter episodes over the northern African continent, increasing the freshwater influx to the Mediterranean. Reduced surface water salinity in the eastern Mediterranean Sea in turn prevented deep-water mixing, resulting in bottom-water anoxia, which in concert with increased organic matter production, resulted in the deposition of so-called sapropels (De Lange et al., 2008; Fontugne and Calvert, 1992; Rossignol-Strick et al., 1982). These orbitally paced events are clearly marked in the sediment as dark, organic-rich layers. Although a substantial body of work provided insights into sapropel formation (see e.g. Rohling et al., 2015 and references therein), it is still a matter of debate how much surface salinity dropped during these intervals, at which thresholds sapropels developed, and the

Abstract

Orbitally controlled hydrological changes in the Eastern Mediterranean display a complex interplay of different latitudinal climate processes. These climatic signals involve the low-latitude monsoon system, the mid-latitude local evaporation-precipitation balance (impacted by high-latitude ice-sheet variability through sea-level variability), and high-latitude northern-hemisphere cold spells affecting local thermohaline circulation. This hydrological variability, in concert with increased organic matter production, has led to regular seafloor anoxia and the deposition of so-called ‘sapropels’, dark-organic rich layers in the marine sediment record. However, the exact triggers of sapropel onset and termination remain debated, whereby the relative influence of Nile outflow and concomitant decrease in surface salinity as well as changes in seawater temperature may both have enhanced stratification. The relative contribution of these controlling processes may differ between sapropels, for example for sapropels deposited under glacial and interglacial conditions. We here compare sea surface temperature and salinity variability for ten sapropels in the Eastern Mediterranean over the past 350 kyr, by applying novel proxies based on element incorporation into foraminiferal calcite. Estimates of Nile river outflow (Ba/Ca, Mn/Ca), surface salinity ($\Delta Mg/Ca$, Na/Ca) and $\delta^{18}O_{\text{water}}$ are compared to established sediment geochemistry proxies recording monsoon strength (Ti/Al), productivity (Ba/Al) and bottom water deoxygenation (Mo/Al). Our results show that both changes in temperature and Nile-river runoff play a consistent role in sapropel onset and termination, albeit with variable amplitudes between sapropels.

relative role of river runoff versus the local evaporation/precipitation balance. As eastern Mediterranean $\delta^{18}\text{O}$ records reflect global ice volume, river run off, basin evaporation, and temperature (Grant et al., 2016), deconvolving these parameters remains complex. Independent estimates of salinity and Nile-river input into the Mediterranean Sea would facilitate isolating the role of the African monsoon system and evaporation on salinity and hence understanding sapropel formation. Here we aim to use several novel proxies based on foraminiferal calcite chemistry to provide such independent estimates of river runoff and surface-water salinity in the Eastern Mediterranean Sea.

Element incorporation in foraminiferal calcite provides a tool for the reconstruction of a wide range of environmental parameters. Besides the more established Mg/Ca ratio, which is used as a paleothermometer (Lea et al., 1999; Nürnberg et al., 1996), multiple novel proxies for seawater salinity, based on trace element incorporation, have been developed over the last decennium. Recent additions to the foraminifera 'proxy-toolbox' are Ba/Ca, reflecting (Nile) river input (Weldeab et al., 2007; Weldeab et al., 2014), Mn/Ca, recording terrestrial Mn input (Klinkhammer et al., 2009) and Na/Ca, potentially providing an independent salinity estimate (Geerken et al., 2018; Mezger et al., 2016; Wit et al., 2013).

Reconstructing sea surface salinities for sapropels include combining Mg/Ca and stable oxygen isotopes (Elderfield and Ganssen, 2000) and $\text{U}^{K'}_{37}$ with the δD (van der Meer et al., 2007). These studies suggest salinity values during Holocene sapropel S1 of about 34 and much lower values during deposition of Eemian sapropel S5. Using Ba/Ca as a qualitative tracer (Weldeab et al., 2014) showed that Nile river-runoff was greatly enhanced during the African Humid Period, and hence sapropel S1. Foraminiferal Ba/Ca reflects seawater Ba concentration (Hönisch et al., 2011; Lea and Spero, 1994) and therefore salinity in estuaries, as riverine runoff is high in dissolved Ba (Weldeab et al., 2007, 2014), but not necessarily salinity as such. Although calibrations for three major estuaries studied are rather similar (Weldeab 2007), estuary-specific calibrations are still required due to differences observed. Unfortunately, a Nile river Ba/Ca-salinity calibration cannot be provided for paleo-reconstructions, as the construction of the Ashwan Dam in 1960 currently limits Nile discharge to a fraction of its original quantity, and only qualitative estimations can be made.

In river water also other elements might be present at higher concentrations compared to seawater, which in case they are incorporated in foraminiferal calcite could act as runoff proxies. In well-oxygenated seawater Mn/Ca is relatively low, at nanomolar levels, whereas (Nile-)river discharge has Mn concentrations of 2.9 $\mu\text{g/L}$ (Dekov et al., 1997). Culture studies have shown that Mn incorporation is linearly related to Mn concentrations of the culture medium (Barras et al., 2018; Munsel et al., 2010). This

relationship has been confirmed for planktonic foraminifera (Steinhardt et al., 2014) and hence foraminiferal Mn/Ca could also provide a potential proxy for past seawater Mn/Ca ratios and for the Mediterranean Nile discharge.

Na is one of the main components of seawater salinity and its uptake into foraminiferal calcite would therefore potentially be an ideal salinity proxy, analogous to inorganic studies showing that Na-uptake in (natural) calcites is positively correlated to salinity (Ishikawa and Ichikuni, 1984; Kitano et al., 1975). First culture and plankton-tow studies indeed showed increased Na-incorporation at higher salinity (Allen et al., 2016; Geerken et al., 2018; Mezger et al., 2016; Wit et al., 2013). Nonetheless, more recently it was found that the positive relationship between Na/Ca and salinity observed in plankton-tow and culture studies was not observed for core-top studies. For spinose-planktonic species this was attributed to the loss of Na-enriched spines (Mezger et al., 2018) which implies a major impact of calcite preservation on foraminiferal Na/Ca records. Still, when preservation of these foraminifera would be excellent, foraminiferal Na incorporation might still yield valuable information.

Foraminiferal Mg/Ca, although primarily affected by temperature, is also affected by salinity (Dueñas-Bohórquez et al., 2009; Gray et al., 2018; Hönisch et al., 2013). Temperature reconstructions in restricted basins hence require a correction for salinity to prevent a major bias, as these areas often experience pronounced changes in salinity over time (Boussetta et al., 2011; Hönisch et al., 2013). Calibration studies, however, yield different sensitivities to salinity, with the most recent compilation studies reporting a 4% increase in Mg/Ca for an increase of 1 salinity unit (Gray et al., 2018), with sensitivities potentially being even higher in saline (Ferguson et al., 2008; Mathien-Blard and Bassinot, 2009). Additionally, in evaporative basins such as the Mediterranean Sea, high-Mg overgrowths may compromise a primary Mg/Ca signal (Boussetta et al., 2011; Hoogakker et al., 2009; Kontakiotis et al., 2011) particularly at salinities above 38.5 (Boussetta et al., 2011). An X-ray diffraction study has shown that high-Mg overgrowths may still affect foraminiferal Mg/Ca values despite a pristine appearance of the shell surface (Boussetta et al., 2011). Presence of high-Mg overgrowths is furthermore found to be heterogeneous within samples and within specimens, and not unambiguously correlated to salinity or bottom water $[\text{CO}_3^{2-}]$ (Boussetta et al., 2011). Although current salinities in the Eastern Mediterranean are approximately 39 and foraminiferal samples are hence prone to high Mg overgrowths, Mg/Ca ratios of foraminifera from sapropels are likely less affected by high Mg-overgrowths. Therefore, we here test the use of 'excess-Mg/Ca' as a proxy for salinity, after correcting the Mg/Ca signal for temperature, while carefully evaluating the presence of high-Mg overgrowths and impact of cleaning protocols.

We here test Ba/Ca, Mn/Ca, and Na/Ca as tracers for Nile river runoff and compare changes therein with the foraminiferal $\delta^{18}\text{O}$ record. Furthermore, we applied these proxies based on element incorporation down-core, to test their applicability in an evaporative basin. Results for river runoff and salinity estimates are compared to established sediment geochemistry proxies, reflecting monsoon intensity (Ti/Al), sea surface productivity (Ba/Al) and bottom water anoxia (Mo/Al). This multi-proxy reconstruction of salinity and hydrology changes in the Eastern-Mediterranean Sea aids understanding the relative importance of river-runoff-induced changes in salinity versus evaporation. Furthermore, results provide insights into the potential of foraminiferal calcite based proxies.

5.2 Material and Methods

5.2.1 Piston core material and sampling

Piston core 64PE406-E1 (Fig. 1, 33° 18.14898' N, 33° 23.71998' E; 1760 m water depth, total core length of 9.6 m) has been retrieved during the Eastern Mediterranean NESSC (Netherlands Earth System Science Centre) cruise. Sediment samples were obtained at a 2 cm resolution throughout the core, and at a 1 cm resolution over sapropels and transitions, to capture changes in salinity at a higher temporal resolution. The sediment samples were freeze-dried and subsequently wet-sieved using Endecott stainless-steel sieves with mesh sizes of 63 and 150 μm after which the samples were dried at 60 °C overnight.

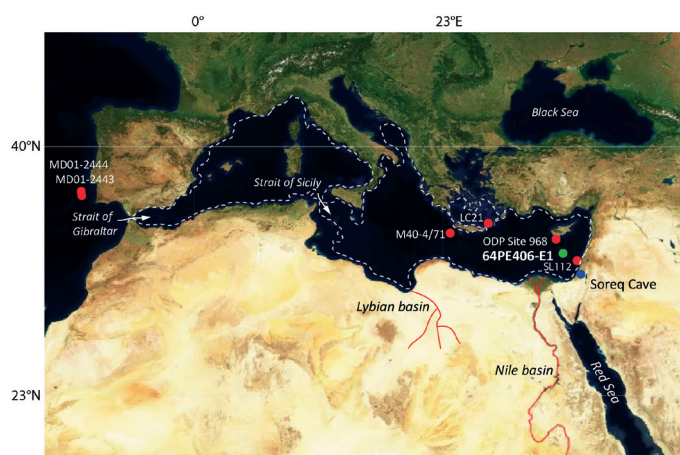


Figure 1. Map showing location of piston core 64PE406-E1 with a green dot, the Nile and Lybian basin that drain into the Mediterranean in red, the LGM sea level in white dashed lines (Mazza et al., 2013), indicating the restricted access of North Atlantic water at the strait of Gibraltar and strait of Sicily during glacial low-stand. Other cores relevant to this study are shown with red dots: SL112 (Weldeab et al., 2014), M40-4/71 (Emeis et al., 2003), MD01-2444 and MD01-2443 (Martrat et al., 2007), ODP Site 968 (Ziegler et al., 2010) and LC21 (Grant et al., 2012) and the Soreq Cave (Grant et al., 2012) is indicated with a blue dot.

5.2.2 Foraminifera sampling and cleaning protocol

Globigerinoides ruber was generally abundant throughout the core, except for interval 487.5 – 508.5 cm (~167-179 kyr BP; corresponding with the sapropel S6 interval), where *G. ruber* was absent, which has been attributed to failure to adapt to adverse conditions (Ganssen and Troelstra, 1987). Between 30 and 40 specimens of *G. ruber* (white) were picked from the 250-355 μm size fraction of each sample, thereby avoiding specimens with a contaminated or encrusted appearance. As signs of diagenetic alteration are not always recognizable using light-microscopy, representative specimens from different core-depths, both sapropel and non-sapropel samples, were assessed for preservation using a tabletop Hitachi TM3000 Scanning Electron Microscope.

Specimens were cleaned using an adjusted Barker protocol (Barker et al., 2003, modified by Cambridge and NIOZ). First, foraminiferal shells were gently crushed between two glass plates, in order to remove contamination such as clays and coccolithophores within the shells during subsequent cleaning steps (Boyle and Keigwin, 1985). The crushed foraminifers were then transported to acid-leached 500 μl -vials. Clays and coccolithophores were removed using a *minimal settling technique*, by removing the supernatant containing the contamination with double de-ionized water and methanol, right after the heavier carbonate shell fragments are settled (~5 seconds). Organic matter and associated contamination was removed using an oxidative cleaning step, by adding an alkali-buffered H_2O_2 solution heated for 10 minutes in an ultrasonic bath at 95 °C. NaOH was hereby replaced by NH_4OH to avoid contamination of the foraminiferal Na/Ca measurements. The oxidizing reagent was removed by several rinses with double de-ionized water. For the oxygen isotope analysis, this was the final step of the cleaning protocol. The samples for the solution-ICP-MS analysis were transferred to new acid-leached vials and required further cleaning steps for the removal of metal (Mn-Fe) oxides and adsorbed ions. An ammonium citrate hydroxide solution with hydrazine hydroxide was added to each sample and placed in an ultrasonic bath for 30 minutes at 95°C and ultra-sonicated 2 seconds every 2 minutes to enhance the reaction. The supernatant solution was removed with several rinses of cold and hot double de-ionized water. Finally, the samples were briefly leached with 0.001 M HNO_3 to remove any adsorbed ions from the foraminiferal shells.

5.2.3 Stable isotope analysis

Stable isotopes of crushed *G. ruber* shells were analysed using a Kiel-IV device coupled to a Thermo-Finnigan MAT253, for a total of 387 samples of 15-45 μg carbonate. A standard run consisted of 38 samples interspersed with 5 in-house standards (NFHS-1) and 4 international carbonate standards (NBS-19). The isotope ratios are given relative

to the Vienna Pee Dee Belemnite (VPDB) standard using the delta notation. Results were normalized against NBS19 ($\delta^{18}\text{O} = -2.20\text{‰}$, $\delta^{13}\text{C} = 1.95\text{‰}$) and in-house standard NFHS-1 ($\delta^{18}\text{O} = 1.148\text{‰}$, $\delta^{13}\text{C} = 0.854\text{‰}$). The mean external reproducibility was better than $\pm 0.05\text{‰}$.

5.2.4 SF-ICP-MS and foraminiferal elemental analyses

Cleaned foraminifera fragments were dissolved in 0.5ml 0.1 M HNO_3 , ultra-sonicated for 15 minutes and centrifuged for 5 minutes to settle any remaining silicate particles. Trace element/Ca ratios were measured for in total 435 samples at the Royal Netherlands Institute for Sea Research (NIOZ) using a SF-ICP-MS (Thermo Fisher Scientific Element-2), measuring ^{23}Na , ^{24}Mg , ^{25}Mg , ^{27}Al , ^{43}Ca , ^{48}Ca , ^{55}Mn , ^{56}Fe , ^{88}Sr and ^{138}Ba . Six ratio calibration standards with similar matrix were used for optimal accuracy and precision of the instrument. After five samples a drift and monitor sample was measured. Quality of the samples was checked with monitor standards JCT1, JCp1 and in-house foraminifera standard (NFHS-1). Trace element/Ca ratios were calculated using MATLAB and were corrected for instrument and dissolution-acid blank.

Measurements with ^{43}Ca counts $< 1\text{e}7$ mmol/mol were excluded from further analysis, as this indicates the shell is not properly ablated. Outliers of whole distribution are removed per element based on statistical test (Appendix A). Foraminiferal Al/Ca, Fe/Ca and Mn/Ca were used to validate the cleaning efficiency of the procedure and contamination by Al rich clay minerals and Mn-Fe coatings (Barker et al., 2003; Appendix A; Pena et al., 2005; Weldeab et al., 2006). Samples with $\text{Al} > 0.4$ mmol/mol and $\text{Fe} > 0.1$ mmol/mol concentrations are considered contaminated (Barker et al., 2003), fitting with values of our statistical outlier analysis ($\text{Fe/Ca} > 0.12$, 12% and $\text{Al/Ca} > 0.31$, 13%) were excluded from further analysis). Mn/Ca is generally much lower in ‘open-ocean’ specimens compared to values observed here, due to low Mn concentration in the seawater, whereby $\text{Mn/Ca} > 0.1$ mmol/mol is usually considered contaminated by MnCO_3 overgrowths (Boyle), Mn-Fe coatings or kutnahorite phases, also high in Mg (Pena et al., 2005, Steinhardt et al., 2014). However, in our samples Mn is not related to Fe, excluding the presence of kutnahorite phases, while Mn is also not related to Al, excluding clay detritus. Furthermore Mn is low in the bulk sediment during sapropels, when foraminiferal Mn/Ca is high, which suggests that high foraminiferal during sapropels not related to sedimentary Mn concentrations, except for a few outliers (Appendix A2). Moreover, the setting we study here is prone to intense recycling of redox sensitive trace metals, which could temporarily increase the concentrations of these metals in the water column. Therefore, $\text{Mn/Ca} > 0.32$ mmol/mol (5%) were considered as outliers based on the distribution statistics (Appendix A). For Ba/Ca measurements with values higher than $5.58 \mu\text{mol/mol}$ were removed and the

signal was smoothed, using the MATLAB smooth function, to better enable comparison to other proxy patterns. Of all measured data, $\sim 22\%$ was rejected because of low ^{43}Ca counts, and from this dataset between 26% en 32% was rejected exceeding threshold values for Al-, Fe-, Mn- combined, Ba- analysis and or Mg/Ca ratios (depending on the element, as for the Ba-analysis more outliers were removed).

5.2.5 LA-ICP-MS and SEM analysis

We assessed the presence of inorganic overgrowths on *G. ruber* Mg/Ca ratios down-core and the efficiency of the cleaning protocol, by analyzing ten specimens from ten intervals (sapropel and non-sapropel periods) using LA-ICP-MS and SEM analysis. In the first analysis specimens were cleaned using an oxidative cleaning step. In the second run, specimens were cleaned similarly as for the SF-ICP-MS analysis, including a methanol and reductive cleaning step (see section 2.3). Prior to laser ablation, SEM images of a total of 37 *G. ruber* specimens were taken to assess the structure of the shell surface and screen for inorganic calcite or other overgrowths (see Appendix B for examples and for comparison Hoogakker et al., 2009 Kontakiotis et al., 2011). The element concentrations of the final 1-3 chambers of each specimen were measured using LA-ICP-MS (see e.g. Mezger et al, 2018 for details). The laser ablation system (NWR19UC, New Wave) consists of an ARF excimer laser with 193 nm pulse width. Each sample was targeted by a laser fluence of $1 (\pm 0.05) \text{ J/cm}^2$ and at a repetition rate of 4 Hz, 2 minutes ablation time and a spot size of $80 \mu\text{m}$. The ablated material was transported in a helium environment (0.7 L/min gas flow) to the ICP-MS (Thermo Scientific iCAP-Q) using a smoother optimized for high sensitivity of ^{24}Mg and low ThO/Th ratios ($< 1\%$).

5.2.6 Bulk sediment elemental composition

The geochemical composition of piston core 64PE406-E1 was obtained through Multivariate Log-ratio Calibration (MLC; Weltje et al., 2015) of X-ray Fluorescence (XRF) core scanner analyses. Details are described in Bale et al. (In Review). In short, an Avaatech XRF core scanner with state-of-the-art detector (Rayspec cubed SiriusSD silicon drift) was used to measure elements Al to Ba. Here, the elements of interest (Al, Ti, Mn, Mo, and Ba) were measured using the settings for trace element analyses (Hennekam et al., 2019): 20 kV with Al primary beam filter (Al, Ti, Mn) and 50kV with Cu primary beam filter (Mo and Ba). Daily measurements of standard material indicated precision (relative standard deviations) $< 10\%$ for all elements. MLC-calibration was performed with the AvaXelerate software (Bloemsma, 2015; Weltje et al., 2015). The 40 sediment samples used for calibration were totally digested with an HClO_4 - HNO_3 -HF acid mixture, evaporated to near dryness, and subsequently dissolved in a HNO_3 (1 N) solution. Subsequently, analyses were done through inductively coupled plasma mass

spectrometry (Thermo Finnigan Element II), with accuracy >7% and precision >2% for all elements of interest. We present elements normalized in a ratio to Al to avoid closed-sum effects through variability in carbonate content (Thomson et al., 1999).

5.2.7 Core chronology

The 64PE406-E1 chronology is based on the well-dated sapropel boundaries (see Hennekam et al., in review for details). However, here the age model is updated through cross-comparison of the $\delta^{18}\text{O}$ of *G. ruber* to well-dated cores (MS21 and LC21; Grant et al., 2012; Zwiép et al., 2018, respectively) and by correlation to the Soreq cave $\delta^{18}\text{O}$. In general, sapropel boundaries seem to be synchronous within the Ionian and Levantine Seas and, hence, these sapropel boundaries in core 64PE406-E1 were linked to the best dates currently available, i.e., Grant et al. (2016) for sapropels S1 to S5 and Ziegler et al. (2010) for Sapropels S6 to S'. The sapropel excursions were aligned using the Ba/Al profiles (and verified using Ti/Al variability), and here we added three tie-points for the deepest part of the core (to sapropel S10) to extend the record to ~350 kyr BP (Appendix C). Moreover, the Holocene part was now covered by adding data obtained for the multicore, as the upper part of the piston core stops at ~10 kyr BP (containing only the last 2.3 cm of sapropel S1 at the top), while the multicore contains the interval from ~10-0 kyr BP.

The here presented $\delta^{18}\text{O}$ record of *G. ruber* allows to further fine tune our existing age model for the interval from 0-130 kyr BP. Following the reasoning of Grant et al. (2012), *G. ruber* $\delta^{18}\text{O}$ correlates well to Soreq speleothem $\delta^{18}\text{O}$ as both are affected by $\delta^{18}\text{O}$ changes of Mediterranean surface waters. Hence, two tie-points were added between 64PE406-E1 *G. ruber* $\delta^{18}\text{O}$ to the Soreq $\delta^{18}\text{O}$ at ~68 and ~55 kyr BP (Fig. 3). Moreover, *G. ruber* $\delta^{18}\text{O}$ records from the Eastern Mediterranean Sea region show similar variability with almost identical absolute values outside sapropel intervals (see e.g. Rohling et al., 2017). This is also the case for core 64PE406-E1, and therefore we added five tie-points in the interval from 0-44 kyr between our piston core and well-dated (through ^{14}C and tephra) cores MS21 and LC21 (Appendix C). The multicore was dated using well-documented basin-wide events (i.e., the end of sapropel S1 at ~6.1 kyr BP (Grant et al., 2016) and the sapropel interruption at ~8.2 kyr BP (Wildeab et al., 2014), while the age of the multicore base was constraint by comparison of *G. ruber* $\delta^{18}\text{O}$ to that of core MS21 (Appendix C).

5.2.8 Proxy interpretation for bulk-sediment geochemistry

Sediment Ti/Al has been shown reflect African monsoon intensity (Lourens et al., 2001; Ziegler et al., 2010), particularly in distal marine sediments (Lourens et al., 2001; Wehausen and Brumsack, 2000). Aeolian input from the African continent is high in Ti

relative to Al, whereas riverine input is high in Al relative to Ti. During intervals of weaker North African monsoon intensity, drier conditions generate transport of aeolian Ti into the Mediterranean Sea, while intervals of stronger monsoon intensity results in increased Al input from the Nile river. Our Ti/Al record appears to reflect both processes involved, as the core site is both within reach of aeolian and riverine sediment input (Fig. 3).

Sedimentary barium enrichments relate to biogenic Ba (BaSO_4) precipitation in the water column, which is formed as organic matter is being decayed during its settlement to the seafloor. Hence, Ba/Al is thought to be a proxy for export-productivity (e.g. Bishop, 1988; Dymond and Collier, 1996), which works particularly well for Mediterranean sapropels (Van Santvoort et al., 1996). Therefore, Ba/Al is here used to mark sapropel intervals (Fig. 3). Sedimentary Mo increases as it is scavenged the presence of H_2S (i.e., euxinia). Hence, higher Mo concentrations are related to euxinia in bottom- and/or pore waters (Helz et al., 1996).

Sedimentary manganese is low during sapropels as solid Mn-oxyhydroxides are reductively transformed into dissolved Mn^{2+} . Sedimentary Mn is only diagenetically enriched at (1) the end of sapropel formation when the water column gets re-ventilated and pore water oxygen can subsequently diffuse downwards into the sapropel sediments (Mn peak marking the top of sapropels), or (2) during sapropel deposition when downward diffusing Mn^{2+} meets oxygen still present in the pore spaces in the sediments below (i.e. Mn peaks below sapropels, see e.g., Van Santvoort et al., 1996; De Lange et al., 2008). Our Mn/Al record mainly shows the latter, with high Mn/Al values at the onset of sapropels S1, S4 and S5 (Appendix A). However Mn/Al is low within sapropel intervals, preventing diagenetic Mn enrichment, which might otherwise have raised our foraminiferal Mn/Ca values. Hence no correlation is observed between sediment Mn/Al and foraminiferal Mn/Ca after removal of few Mn/Ca outliers (Appendix A). These outliers in foraminiferal Mn/Ca are associated with Mn fronts at the onset of some sapropels, and therefore have been omitted from further consideration. Our sediment geochemistry results hence provide the framework to validate our foraminiferal carbonate-based proxies for their climate derived signals as well as potential diagenetic overprinting.

5.2.9 Excess-Mg calculation

Applying the most widely applied temperature calibrations (Anand et al., 2003) to our Mg/Ca record results in unrealistically high temperature estimates. During sapropels however, values are more comparable to 'normal' open ocean foraminiferal Mg/Ca values of around 3 mmol/mol (Fig. 2). Our Mg/Ca record can hence not be readily applied as a paleo-thermometer and is likely more strongly affected by salinity, possibly also related

to high Mg-overgrowths. Boussetta et al., 2011, did observe high-Mg overgrowths on the outside of foraminiferal shells from the Eastern Mediterranean using X-ray diffraction (XRD), but used an ‘oxidative protocol’ (Barker et al., 2003) only. This protocol is less efficient in removing high Mg-overgrowths, compared to the ‘reductive protocol’ applied here (Pena et al., 2005). Still, our *G. ruber* specimens do not show signs of inorganic overgrowths using Scanning Electron Microscopy (SEM), before or after applying the reductive cleaning step (Appendix B). Before the reductive cleaning step, specimens from high-Mg/Ca intervals samples were encrusted with coccolithophores, and show high Al/Ca peaks at the outside of the shell coinciding with high Mg/Ca peaks (Appendix B). After applying the reductive cleaning protocol these peaks were greatly reduced, and the Mg/Ca signal of ‘high-Mg’ specimens appears high throughout the shell-wall (Appendix B). Furthermore, all SF-ICP-MS analyses are low in Al/Ca values and there is no correlation between Mg/Ca and Al, Fe or Mn (Appendix A), indicating that within our solution data high Mg/Ca values are not related to Al/Ca peaks or Mn-Fe-rich overgrowths. Nevertheless, as it was not feasible to scan all individual specimens used for the solution analyses with SEM we cannot fully exclude that samples showing high-Mg/Ca values are influenced by high-Mg overgrowths.

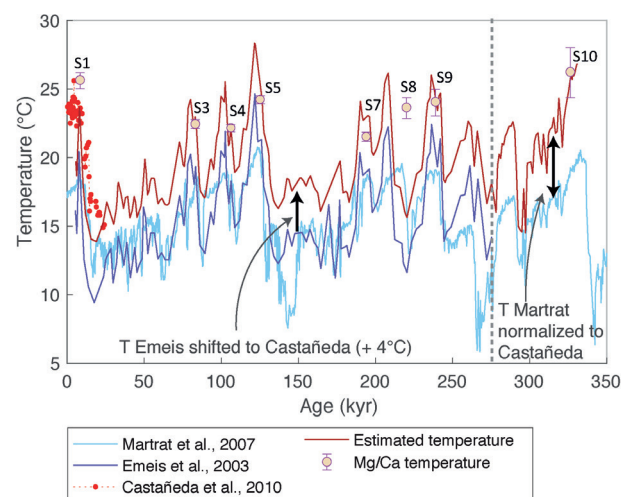


Figure 2. Purple dots indicate the *G. ruber* Mg/Ca-derived temperature estimates for Sapropel intervals based on uncorrected Mg/Ca values using the Anand et al., 2003 equation for *G. ruber* white (250–350µm fraction). Purple lines indicate the Mediterranean Ridge $U^{k'}_{37}$ -temperature record (core M40-4/71, Emeis et al., 2003) which was shifted 4° up resulting in a temperature estimate for the first ~275 kyrs of our record (red line), to fit an alkenone-derived temperature record from the Eastern Mediterranean covering the last 27 kyrs (red dots, Castañeda et al., 2010). The temperature reconstruction for 275–350 kyrs (red line) was obtained by normalizing a $U^{k'}_{37}$ -temperature record from the Iberian margin which covers the last ~400 kyr (Martrat et al., 2007) to the same alkenone-derived temperature record (Castañeda et al., 2010).

Boussetta et al., (2011) provide a calibration for ‘excess-Mg/Ca’, defined as measured Mg/Ca minus expected Mg/Ca based on temperature (hereafter: $\Delta\text{Mg/Ca}$). This $\Delta\text{Mg/Ca}$ shows a linear relationship to salinity based on a compilation of various core top studies, in line with a calibration already proposed by Mathien-Blard and Basinot (2009):

$$\text{Salinity} = \frac{\Delta\text{Mg/Ca} + 23.97}{0.67} \quad \text{Equation 1.}$$

Above a salinity of 38.5 this relationship is considered unreliable due to heterogeneous and unpredictable occurrence of inorganic overgrowths (Boussetta et al., 2011). Since we cannot exclude that high Mg/Ca overgrowths have affected our Mg/Ca values during more saline periods, we consider $\Delta\text{Mg/Ca}$ between >2 as speculative. On top of this it has to be noted that it has been recently suggested that Mg/Ca is also affected by the seawater carbonate chemistry (pH or $[\text{CO}_3^{2-}]$, Gray et al., 2018), for which we here cannot correct. Hence, our $\Delta\text{Mg/Ca}$ -derived salinity estimate is semi-quantitative at best.

Although a local temperature record would be highly preferred to derive $\Delta\text{Mg/Ca}$, there is no temperature record covering the entire time interval we here investigate for the Eastern Mediterranean. We therefore estimated temperature by shifting a Mediterranean Ridge $U^{k'}_{37}$ -temperature record (Emeis et al., 2003) with 4°, to fit an alkenone-derived temperature record from the Eastern Mediterranean covering the last 27 kyrs (Castañeda et al., 2010) for the first ~275 kyrs of our record. A temperature reconstruction for 275 to 350 kyrs was obtained by normalizing a $U^{k'}_{37}$ -temperature record from the Iberian margin, which covers the last ~400 kyr (Martrat et al., 2007), to the same alkenone-derived temperature record (Castañeda et al., 2010). Fig. 2 shows *G. ruber* derived Mg/Ca temperatures per sapropel interval, using the Anand et al., 2003 equation for *G. ruber* white 250–350µm fraction, and are reasonably comparable to the reconstructed temperature record as these samples are least biased by high salinities (Fig. 2). We used the reconstructed temperature record (Fig. 2) to calculate $\Delta\text{Mg/Ca}$ from measured Mg/Ca and subsequently convert this to salinity estimates (equation 1, Fig. 6). We emphasize once more that the resulting salinity estimate is based on a specific calibration for the Mediterranean by Boussetta et al., (2011) and Mathien-Blard and Basinot (2009), but is semi-quantitative. When applying this calibration, sapropel samples appear to reflect feasible salinities, with values as low as 33–34 within sapropel intervals, comparable to salinity estimates based on e.g. δD for S5 (van der Meer et al., 2007).

5.3 Results

5.3.1 Core geochemistry

Core 64PE406-E1 shows geochemical variability related to sapropel formation (Calvert and Fontugne, 2001) with (1) high Ba/Al during sapropels and lower values in between, (2) lower Ti/Al values during sapropels and higher in between, (3) low Mn/Al during sapropels with sometimes high peaks above sapropels and below, (4) higher Mo/Al values during sapropels and lower values in between. Noteworthy is the higher Ba/Al peak in between sapropels S5 and S6 that co-occurs with lower Ti/Al values and more negative *G. ruber* $\delta^{18}\text{O}$ values (Fig. 3). To better assess differences in proxy-signals between sapropel and non-sapropel periods, we divided the record into 'Sapropel' and 'non-Sapropel' samples, based on the Ba/Al record (threshold value of ~ 0.003 - 0.004 , excluding the Ba enrichment between S5 and S6, and somewhat depending on the base line values just before sapropel times, see Fig. 3). The higher Ba/Al values between sapropel S5 and S6 might indicate sediments previously characterized by high organic carbon and sea, but which are now oxidized, defined as "Ghost" sapropels (as defined in Rohling et al., 2015). Alternatively, conditions at that time never resulted in seafloor anoxia and deposition of a sapropel, although this would be expected based on orbital forcing and may have tended towards sapropel conditions (i.e., higher productivity in this case). Currently, we cannot distinguish between these two options, but we will refer to the high Ba/Al layer between S5 and S6 as a 'missing sapropel'.

5.3.2 Stable isotopes

The stable oxygen isotope values of *G. ruber* in this record vary between -2 to 3 permille. *G. ruber* $\delta^{18}\text{O}$ values are significantly more depleted within sapropels compared to the non-sapropel intervals (Kruskal-Wallis test, $p < 0.0001$, $\text{Chi}^2 = 140$). $\delta^{18}\text{O}$ values are paced by both glacial-interglacial changes in ice volume, but also show large variability on a precessional scale, with values being more depleted during these sapropels. The most depleted values are associated with the onset of sapropels, gradually become heavier again towards the end (Fig. 3).

5.3.3 Whole shell chemistry

All foraminiferal element to calcium ratios show clear precession cycles (Fig. 3). Mn/Ca and Ba/Ca show a strong resemblance to the $\delta^{18}\text{O}$ signal (Fig. 3). Moreover, Mn/Ca and Ba/Ca are significantly higher in sapropel compared to non-sapropel intervals (Fig. 4). Mg/Ca shows a different pattern, with high peaks in between sapropels, highest values coinciding with precession maxima and hence northern hemisphere summer insolation minima (Fig. 3). Values for sapropel intervals are significantly lower compared to non-sapropel intervals. The down core record for Na/Ca shows much scatter, with no significant difference between sapropels and non-sapropel intervals (Fig. 4).

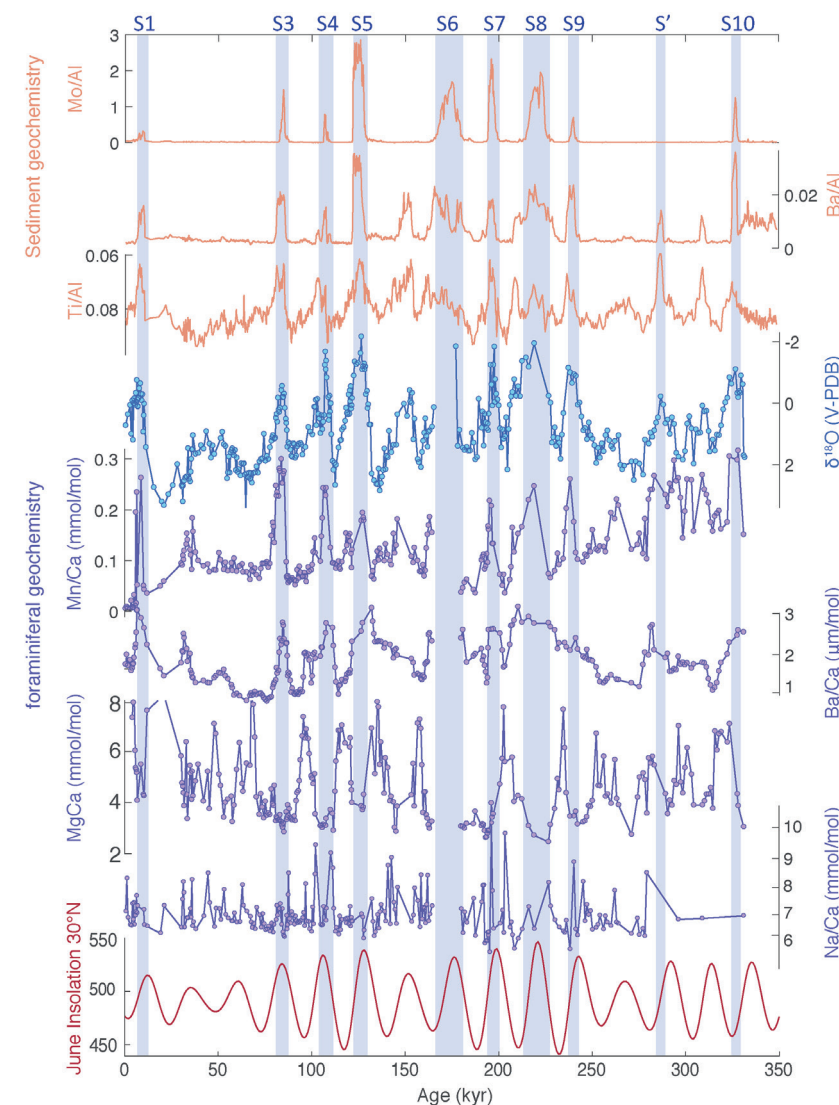


Figure 3. XRF records are shown in pink, with Ba/Al as a proxy for productivity and Mo/Al as a proxy for euxinia, indicating the positions of sapropels. Sapropel nomenclature as defined by Ziegler et al. 2010 is indicated with letters above blue bars, which duration is based on Mo/Al from our study. Ti/Al is a proxy for dust versus river input and thereby monsoon intensity. Proxies based on foraminiferal calcite (*G. ruber*) are plotted in blue, with Mn/Ca as a proxy for seawater Mn concentration, Ba/Ca a proxy for seawater Ba concentration and thereby Nile-river runoff (Weldeab et al., 2007; 2014), and Mg/Ca being influenced by temperature (e.g. Nürnberg et al., 1996), salinity (e.g. Gray et al., 2018) and possibly diagenetic overgrowths (Boussetta et al., 2011, Hoogakker et al., 2009) and Na/Ca as a potential proxy for salinity (e.g. Mezger et al., 2016). $\delta^{18}\text{O}_{G. ruber}$ values primarily reflect $\delta^{18}\text{O}_{\text{water}}$ (global ice volume, river runoff and precipitation) and temperature. Insolation for 30° North is shown in dark red.

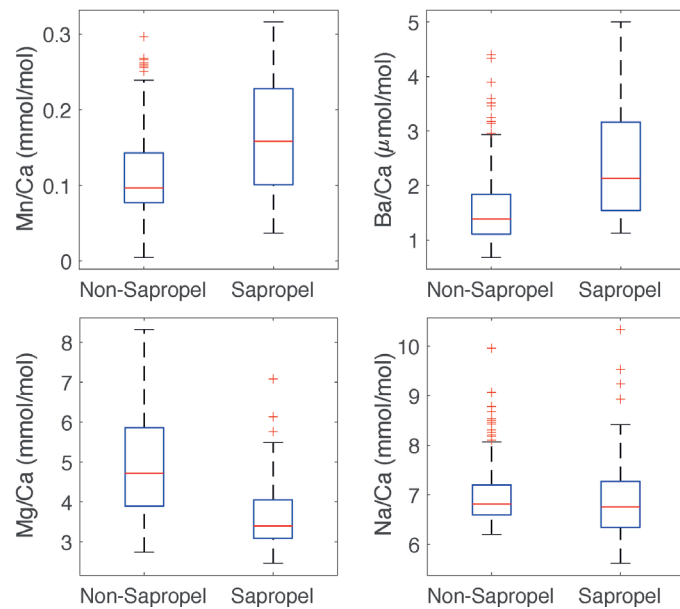


Figure 4. Boxplots for foraminiferal Mn/Ca, Ba/Ca, Mg/Ca and Na/Ca SF-ICP-MS results, grouped for sapropel and non-Sapropel intervals. There is a significant difference between the medians of the sapropel and non-sapropel groups for Mn/Ca, Ba/Ca, Mg/Ca, based on Kruskal-Wallis test ($p < 0.0001$), but not for Na/Ca.

5.4 Discussion

5.4.1 Seawater stable oxygen isotopes

Today, Mediterranean $\delta^{18}\text{O}_{\text{seawater}}$ is strongly imprinted by evaporation and local precipitation (Gat et al., 1996; Pierre, 1999). However, relative impact of both precipitation and evaporation varied greatly in the geological past. Here we provide a high-resolution Eastern Mediterranean $\delta^{18}\text{O}_{\text{G.ruber}}$ record for the last 350 kyr, which includes 10 sapropel intervals. The depleted $\delta^{18}\text{O}_{\text{seawater}}$ values during sapropel intervals are in line with enhanced precipitation and river runoff related to an intensified monsoon (Grant et al., 2016; Rossignol-Strick et al., 1982). During non-sapropel intervals heavy $\delta^{18}\text{O}_{\text{G.ruber}}$ values correspond with high evaporation, similar to present-day values. The here observed large amplitude in $\delta^{18}\text{O}_{\text{G.ruber}}$ also includes changes in global ice volume and hence sea level. As the Mediterranean is an evaporative restricted basin the impact of sea level is enhanced, similar to the Red Sea (Grant et al., 2012, 2016). This implies that of the observed 5‰ variability, 3‰ can be attributed to sea level (Grant et al., 2016), although of these 3‰ only 1.1‰ is directly related to changes in the oxygen isotope inventory of the global ocean due to changes in ice volume. To distract the sea level-effect from the $\delta^{18}\text{O}_{\text{G.ruber}}$ record allowing comparison to Nile-river proxies, residual $\delta^{18}\text{O}$ can be used.

We calculated residual $\delta^{18}\text{O}$ values ($\delta^{18}\text{O}_{\text{residuals}}$), using the method of Rohling et al. (2014) and Grant et al. (2016), to correct for the enhanced glacial concentration effect that occurs in the restricted Mediterranean Sea. In short, we converted the 0.5 Ma long Red Sea sea-level record of (Grant et al., 2014) to equivalent eastern Mediterranean surface-water $\delta^{18}\text{O}$ using the formulas provided in (Rohling et al., 2014), which were normalized to Late Holocene values, and subtracted from $\delta^{18}\text{O}_{\text{ruber}}$. Most negative eastern Mediterranean $\delta^{18}\text{O}_{\text{residuals}}$ values are observed during sapropel intervals (Fig. 6). This suggests, in line with previous literature, that heavily depleted monsoon precipitation via Nile-river discharge was responsible for the stratification of the eastern Mediterranean resulting in sapropel formation. It remains, however, debated how depleted Nile river water actually was during past sapropel times (Emeis et al., 2000). Enhanced tropical precipitation might have resulted in a stronger amount effect over the Nile catchment area and hence a more isotopically depleted fresh water end-member.

A strong impact of precessional paced monsoon variability on the oxygen isotope signal is corroborated by the positive correlation between Ti/Al and $\delta^{18}\text{O}_{\text{G.ruber}}$ ($r^2 = 0.6$), reflecting the alternation of dry (high Ti, high $\delta^{18}\text{O}$) and wet (low Ti/high Al and low $\delta^{18}\text{O}$) periods. Sapropel deposition is hence associated with more humid episodes, although independent river-runoff proxies remain necessary to quantitatively constrain the input of Nile water versus the effect of local changes in evaporation and precipitation.

5.4.2 Fresh water runoff variability

Enhanced Nile-river runoff during sapropels has been recognized for many decades as an important driver for sapropel formation (see Rohling et al., 2015, and references therein). Various proxies have been applied to show enhanced Nile-river outflow during sapropels, such as the oxygen isotope signal of planktonic foraminifera (e.g. Vergnaud-Grazzini et al., 1977), river transported sediments (Revel et al., 2010) and foraminiferal Ba/Ca values (Weldeab et al., 2014). Increased Ba/Ca has also been observed in the central-Eastern Mediterranean during Dansgaard-Oeschger events, but were here attributed to fresh water input from the northern borderlands (Sprovieri et al., 2012). Additionally, fresh water input in the Gulf of Sidra from North Africa has been suggested as an additional source for fresh water input (Osborne et al., 2010; Rohling et al., 2002). Hence the relative importance of Nile-river runoff and other freshwater sources and to what extent this varied between sapropels remains debated.

In general, evidence for a dominant role of Nile input comes from basin-wide trends in $\delta^{18}\text{O}$ signals, showing most depleted $\delta^{18}\text{O}$ levels near the Nile-river estuary (Emeis et al., 2000). We here show that also Ba/Ca was enhanced for every sapropel during the last 350 kyrs, indicating that Nile-river runoff is a consistent factor in sapropel formation. Values

are similar to those observed by Weldeab et al., 2014 for sapropel S1 at a location slightly more remote from the river Nile. The record older than S5 is somewhat less pronounced, which may indicate that the Ba/Ca signal is subject to slow recrystallization (Weldeab et al., 2014). Although this hampers a more quantitative comparison of Nile input between sapropels further downcore, an inverse linear relationship is still observed between Ba/Ca and $\delta^{18}\text{O}$ (Fig. 5). As the average for individual sapropels and non-sapropel samples fall on this trend this suggests a consistent, yet variable, role of Nile input in sapropel formation. Deviating from the general trend are sapropel S5 and S9 (Fig. 5, assuming that $\delta^{18}\text{O}$ is less prone to diagenetic effects), which have lower Ba/Ca. For sapropel S5 Rohling et al. (2002), showed an additional more depleted fresh water source in the Gulf of Sidra (Fig. 1), which may explain a lower Ba/Ca signal due to a different freshwater source. Potentially this also played a role during deposition of sapropel S9.

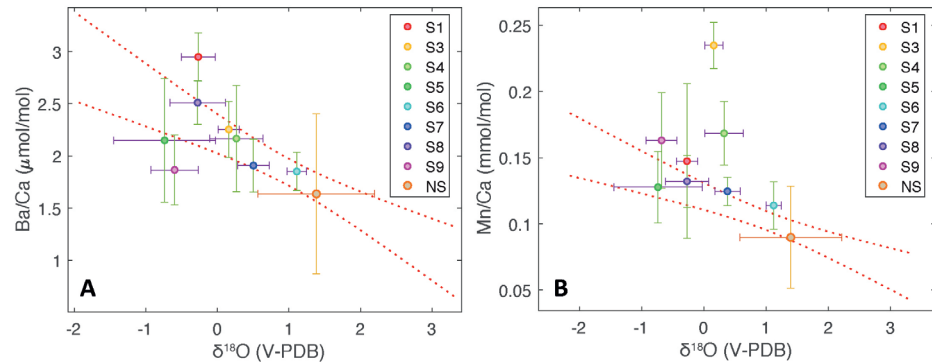


Figure 5. A. Mean Ba/Ca (A) and Mn/Ca (B) against mean $\delta^{18}\text{O}$ per sapropel and the mean for non-sapropel intervals (colored dots, error bars indicate standard error, sapropel number indicated in legend). Dashed lines indicate the 95% confidence interval for relationship between $\delta^{18}\text{O}$ and Ba/Ca, without S5 and S9, $\text{Ba/Ca} = -0.37 * \delta^{18}\text{O} + 2.21$ ($R^2 = 0.16$, $p < 0.001$) and between $\delta^{18}\text{O}$ and Mn/Ca, without S3, $\text{Mn/Ca} = -0.02 * \delta^{18}\text{O} + 0.12$ ($R^2 = 0.14$, $p < 0.001$), assuming that $\delta^{18}\text{O}$ is least affected by diagenetic alteration, hence deviations from the trend are considered to be due to Mn and Ba outliers.

In addition to Ba also Mn is often present in higher concentrations in river runoff, such as the Nile. How far Mn travels in seawater fundamentally depends on the rate at which Mn oxides are formed, but generally Mn can be used as a conservative tracer of freshwater input due to slow Mn oxidation kinetics (von Langen et al., 1997). Uptake of Mn in foraminiferal shell carbonate largely reflects seawater Mn concentration (Barras et al., 2018; Munsel et al., 2010). Foraminiferal Mn/Ca is not often used as a proxy as it is highly susceptible to diagenetic overgrowths. However, more recently several studies have shown that, when using a reductive cleaning step, Mn/Ca potentially provides useful information on past seawater Mn (Koho et al., 2015; Ní Fhlaithearta et al., 2010; Steinhardt

et al., 2014). Since high Mn/Ca values in our record, moreover, coincide with (relatively) low sedimentary Mn values during sapropels (Appendix A) a diagenetic overprint seems unlikely. The foraminiferal Mn/Ca record furthermore shows a high resemblance to the residual- $\delta^{18}\text{O}$ record, with high Mn/Ca corresponding to depleted residual- $\delta^{18}\text{O}$ values (Fig. 6). In the Eastern Tropical North Pacific enhanced *G. ruber* Mn/Ca signals have recently been attributed to increased terrestrial influx of Mn during the last deglaciation and concomitant sea level rise (Klinkhammer et al., 2009). Given the short residency time of Mn in the seawater (~50 years) prolonged episodes of high Mn/Ca, as observed here, cannot be explained by a single fresh water pulse, but suggest sustained inputs of Nile river runoff, which currently contains about 50 nmol/kg Mn (Dekov et al., 1997). From the different sapropels considered here S3 appears to have somewhat high Mn/Ca values, falling above the trend (Fig. 5), which suggests that other sources for Mn might be involved as well.

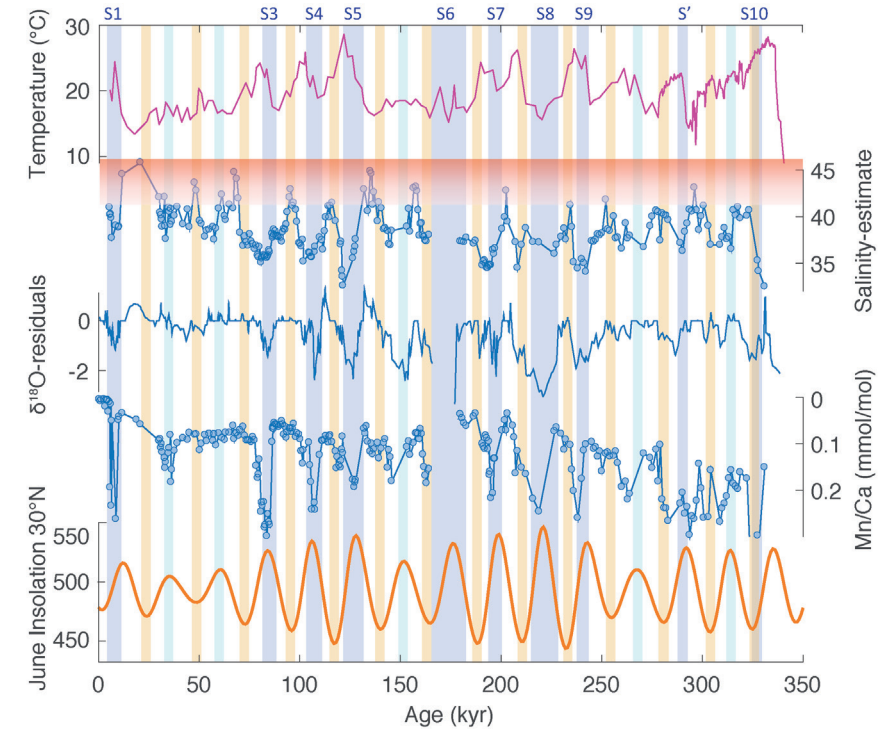


Figure 6. Compilation of the temperature estimation (see Fig. 2 and method section 2.9) in pink, the salinity estimates based on foraminiferal $\Delta\text{Mg/Ca}$ (see method section 2.9) in blue, with a red bar indicating values > 38.5 , above which the relationship between $\Delta\text{Mg/Ca}$ and salinity is deemed unreliable due to potential high-Mg overgrowths (Bousetta et al., 2011). June insolation for 30°N is shown in orange. Sapropels are indicated with dark blue bars. Light blue bars indicate insolation maxima for 'missing sapropels' and yellow bars insolation minima, coinciding with hyper-saline intervals (which may partly be affected by high Mg-overgrowths). Sapropel intervals show low salinities (~33-34) and a high Nile-river influx (reflected by high Mn/Ca values and depleted residual- $\delta^{18}\text{O}$, in blue).

During bottom water anoxia Mn might be remobilized from the sediment and also from dust particles, which often have a Mn-Fe oxide rich coating falling through the water column. Both of these processes could potentially provide an additional source of Mn. However, as *G. ruber* is a surface-dweller, this would require mixing of the oxygen depleted deeper waters with the surface water. During sapropel periods these surface waters are strongly stratified due to the influx of fresh water, but still some mixing might occur, at the same time adding nutrients to the upper mixed layer. The extra Mn incorporated in foraminiferal shell carbonate during deposition of sapropel S3 (Fig. 5) suggest that stratification was somewhat less intense, allowing an additional flux of Mn to the surface water.

During non-sapropel periods, evaporation is expected to shift $\delta^{18}\text{O}$ towards enriched values, but not to affect seawater Mn/Ca and Ba/Ca, which are coupled exclusively to fresh water inputs. This corresponds with the absence of a trend between $\delta^{18}\text{O}$ and Mn/Ca and Ba/Ca during non-sapropel times. Comparing our Nile-runoff proxies to the precession dominated low latitude summer insolation and anoxia proxies, shows that enhanced Nile-runoff is concomitant with bottom water anoxia during phases of amplified precession, in line with earlier studies (Rossignol-Strick, 1985). Comparing the different proxies, it seems possible to “rank” sapropels in terms of amount of Nile river runoff. Although we have to highlight that the shape of the Nile delta has considerably changed over the last 350 kyr (e.g. Ducassou et al., 2009) and thus subtle differences in such a rank may also be due to delta evolution over time. Still, such ranking can give a first impression of Nile intensity in relation to global climate variability. Sapropels 1, 5, 8 and 9 show the highest Nile river runoff reaching the core site, based on $\delta^{18}\text{O}$, Mn/Ca, and Ba/Ca (Fig. 5, except Ba/Ca for 5 and 9 showing an offset from Ba/Ca- $\delta^{18}\text{O}$ relationship). This may be explained by these sapropels falling in the warm, interglacial periods, during which the northern hemisphere summer monsoon is amplified already. Sapropel 3, 4 and 7, coinciding with relatively colder phases (i.e. slow transitions towards full glacial conditions), show less Nile river runoff, suggesting that during colder phases monsoon precipitation and hence Nile river runoff were less enhanced (Fig. 5). S6 is not entirely captured, therefore the average falls in between the other sapropel and non-sapropel values (Fig. 5), yet the base of S6 suggests high Nile influx based on highly depleted (residual-) $\delta^{18}\text{O}$, high Mn/Ca levels, and a relative decrease in Ti/Al (Figs. 3 and 6), which is also supported by data of Emeis et al. (2003).

5.4.3 Changes in sea surface salinity and temperature related to precessional cycles

Although Nile-river runoff was clearly enhanced during sapropel deposition (Rohling, 2015 and references therein and this study), seawater surface salinities during sapropels remain poorly quantified. Hence we here applied a potential new independent proxy for

salinity, Na/Ca (Wit et al., 2013, Mezger et al., 2016, Geerken et al., 2018). However, using calibrations based on culture studies and plankton tow data, converting Na/Ca into past salinity results in unrealistic values. Average Na/Ca values obtained here (~7 mmol/mol) are lower than expected compared to the Red Sea plankton-tow calibration for *G. ruber* (Mezger et al., 2016), yet comparable to ratios from a Red Sea core-top study (Mezger et al., 2018) and a culture study (Allen et al., 2016). Recently such an offset between living foraminifera and core top derived specimens was explained by loss of spines enriched in Na (Mezger et al. 2018). Within sapropels Na/Ca shows higher variability in Na/Ca than expected based on culture studies (Wit et al. 2013, Geerken et al., 2018, Allen et al., 2016), which would be in line with variable preservation of foraminiferal spines and spine-bases. This high variability might be explained by the bottom-water anoxia, which might affect preservation, with Na in spine bases sometimes being better preserved in the sediments. Although at these times bottom waters are corrosive due to high $p\text{CO}_2$, which increases spine (base) loss, limited bioturbation due to bottom water anoxia also results in pore waters becoming rapidly super-saturated with respect to calcite, which enhances preservation. Depending on burial rates preservation and hence Na/Ca is expected to vary greatly at these times. In addition, an overall decrease in Na/Ca over time may be due to lattice diffusion (Yoshimura et al., 2017), although this has been suggested to be relevant mainly on much longer time scales (Myrs time scale). Irrespective of the processes involved, the Na/Ca record presented here seems of limited use for the quantitative reconstruction of past salinity.

Foraminiferal Mg/Ca values in the eastern Mediterranean appear to have a higher sensitivity to salinity (Mathien-Blard et al., 2009 Boussetta et al., 2011) than culture and open-ocean studies, although this difference remains debated (see e.g. Gray et al., 2018, Hönisch et al., 2013). Possibly, the Mg/Ca signal primarily reflects post-mortem addition of inorganic calcite with elevated Mg/Ca. However, inorganic high-Mg calcite overgrowths are characterized by Mg/Ca values one order of magnitude higher (Hoogakker et al., 2009) than values here observed, even in the outer calcite layer before the reductive cleaning step (Appendix B). The SEM pictures (Appendix B) shows that specimens with the highest Mg/Ca values still have clear spine holes. As these holes form during the (partial) resorption of spines, preceding gametogenesis, this implies that the deposition of the ‘higher’-Mg/Ca phase must have occurred when the foraminifer was still living. Post-mortem precipitation would have resulted in these holes being filled up with diagenetic calcite, although possibly overgrowths may occur in small patches as well (Boussetta et al., 2011). However, using laser ablation ICP-MS analyses we verified that Mg/Ca values are elevated throughout the shell wall, and do not reach values associated with inorganically precipitated calcite (Appendix B). Precipitation of

biogenic calcite on foraminiferal tests with a different chemistry, escaping the normal tight organismal control, has been shown previously for deep-dwelling species in the southern Indian Ocean (Steinhardt et al., 2014). Also similarly to earlier observations on living foraminifera, we observe that most of the ‘less organized’ calcite is added close to the aperture, including a hint of striae from the cytoplasm (Appendix B, Steinhardt et al., 2014). Therefore we here propose that the high foraminiferal Mg/Ca values recorded in the eastern Mediterranean reflect a less tight control on Mg incorporation during (bio) mineralization. We hypothesize that this process is enhanced by salinity, so that at higher salinities, the Mg/Ca is elevated. This may serve as an explanation for the previously observed higher sensitivity of Mg/Ca to salinity in the Mediterranean (Ferguson et al., 2008; Mathien-Blard et al., 2009) yet requires further investigation. Irrespective of the mechanism behind the relatively high foraminiferal Mg/Ca values observed here, the $\Delta\text{Mg/Ca}$ signal appears to reflect changes in salinity associated to precessional changes in evaporation-precipitation and Nile-river runoff (Fig. 6). Importantly, the observed increased sensitivity of Mg/Ca to salinity suggests that proxy calibrations should be used with care and only for the appropriate salinity ranges.

The salinity estimate based on $\Delta\text{Mg/Ca}$ suggests that salinities reached values of ~33-34 during sapropel intervals and high salinities similar to today or somewhat higher, coinciding with insolation minima. In comparison to the monsoon runoff related proxies (Ba/Ca, Mn/Ca, Ti/Al, $\delta^{18}\text{O}$), the salinity-estimate record appears somewhat out of phase. This is especially pronounced for sapropel S3, S4, and S5, whereby the onset of low salinity conditions as recorded by $\Delta\text{Mg/Ca}$ precedes the increase in Nile-river runoff and depleted $\delta^{18}\text{O}$ (Fig. 6). This may indicate a non-linear relationship between Nile-river runoff and the local evaporation-precipitation balance, whereby local evaporation may be reduced before the monsoon maximum, resulting in a stronger salinity drop compared to $\delta^{18}\text{O}_{\text{G. ruber}}$. High salinities are recorded in between sapropels, related to insolation minima, yet these intervals show less variability in $\delta^{18}\text{O}_{\text{G. ruber}}$, which may also be explained by increased excess-evaporation during insolation minima (Fig. 6). Additionally, glacial salinity-estimates are high, fitting reconstructed values of 41.5 in the eastern basin during the last glacial maximum of (Thunell and Williams, 1989). Restricted Atlantic inflow during glacial sea level low-stands, in combination with high excess-evaporation may explain these high salinities (Grant et al., 2016).

Another important factor for sapropel formation is temperature, whereby warm sea surface temperatures, especially following cold phases preconditioning the deep-water, may enhance stratification (Emeis et al., 2003). Sapropels occur directly after Northern Hemisphere cold spells, clearly recorded in the Iberian Margin record (Martrat et al.,

2007, Fig. 2), which has been suggested to delay monsoon onset relative to insolation maxima (Ziegler et al., 2010). This temperature drop is likely less pronounced in the Eastern Mediterranean Sea, yet may still affect deep-water formation.

$\delta^{18}\text{O}_{\text{calcite}}$ shows a significant correlation with temperatures with a slope (-0.23, $R^2=0.30$) closely matching the established temperature effect on foraminiferal $\delta^{18}\text{O}_{\text{calcite}}$ of 0.2 degree per ‰, plotted as a series of parallel lines in Fig. 7 (Shackleton, 1974). Simplified, deviations from the average $\delta^{18}\text{O}_{\text{water}}$ trend are related to changes in Nile river runoff (more negative) and/or differences in evaporation and global ice volume effects during glacials (more positive). Dividing the plot of temperature versus $\delta^{18}\text{O}_{\text{calcite}}$ in quadrants shows that most sapropel samples plot in the humid/warm quadrant (Fig. 7A). Fig. 7B thereby shows that also temperature likely played an important role in setting the stage for sapropel formation by increasing buoyancy, as already proposed by Emeis et al., 2003. Sapropels, especially S3, S4 and S5, appear to develop after a rise in temperature, whereby increased Nile-influx likely triggers the onset of sapropel formation (Fig. 7B). Our combined proxy-record suggests that concomitant with the warming trend, enhanced freshwater influx sustained sapropel conditions (Fig. 6). A large part of the $\delta^{18}\text{O}_{\text{G. ruber}}$ variability is already explained by temperature, whereby a rapid increase in Nile river runoff, followed by a gradual decrease, as indicated by Mn/Ca, Ba/Ca and Ti/Al, likely triggered a decrease in overturning circulation. Glacial sapropel S6 thereby seems to have developed, despite overall colder conditions, due to a relative change in temperature and a strong, insolation modulated heavy monsoon, supported by relatively high Nile-river runoff for this interval (Fig. 6). However, a temperature reconstruction from ideally the same record would be required to confidently unravel relative impacts of fresh water inputs versus evaporation/precipitation.

5.4.4 Missing Sapropels at precession minima

During the last Glacial, salinities are relatively low at insolation maxima (36 and 60 kyr). We find slightly higher Mn/Ca, Ba/Ca and lower Ti/Al at 36 kyr. Still, no sapropel sediments were formed, as indicated by Ba/Al and Mo/Al records (and in accordance with many other Mediterranean records). The combined proxy records suggests that this is mainly due to relatively less Nile-river inflow compared to intervals with northern hemisphere summer insolation maxima in interglacial periods. Highly saline conditions and relative cold temperatures probably prevented the sea surface to reach densities low enough to slow down overturning circulation (Fig. 7).

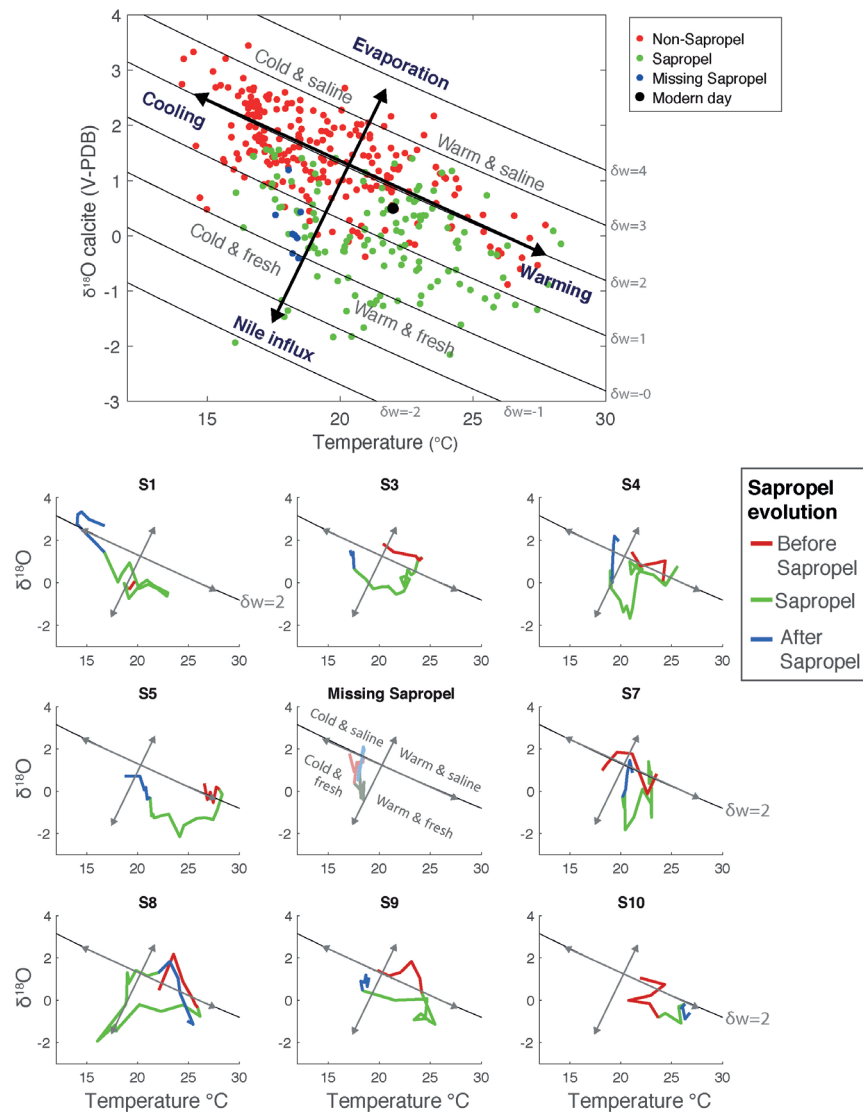


Figure 7. Upper panel: $\delta^{18}\text{O}_{\text{calcite}}$ is plotted against the temperature-estimate, showing a correlation of $R^2=0.3$, with grey lines indicating the conversion to $\delta^{18}\text{O}_{\text{water}}$ using the Shackleton et al., 1974 transfer-function. Moving along this $\delta^{18}\text{O}_{\text{calcite}}$ -temperature trend, e.g. along the $\delta^{18}\text{O}_{\text{water}}$ lines, reflects warming or cooling. Deviations from the temperature-effect on $\delta^{18}\text{O}_{\text{calcite}}$ likely reflect increased Nile influx (more depleted values) and increased evaporation (enriched values), as well as the ice effect (not indicated here). This results in 4 quadrants, reflecting different conditions. Sapropel samples hence fall mainly within the warm & fresh quadrant. Lower panel: When exploring the evolution of sapropels, sapropels are preceded by a warming trend, then show a rapid onset due to fresh water influx and are terminated after a more gradual decrease in the freshwater influx and concomitant cooling, both increasing surface density again (this pattern is mainly clear for sapropel 3, 4 and 5).

A potential “missing sapropel” during the NH- summer insolation maximum at ~152 kyr (stage 6) interestingly shows highly depleted $\delta^{18}\text{O}$ values, high Mn/Ca, Ba/Ca and low Ti/Al, indicating enhanced Nile runoff. Moreover, higher productivity (sediment Ba/Al) seems to have also prevailed during this interval. Still, we find no evidence for a real sapropel (e.g. anoxia indicated by Mo/Al). The salinity estimate does not reach values as low as sapropel intervals (Fig. 6). This may be due to continued mixing with higher salinity seawater during the glacial. Possibly, despite enhanced nutrient inputs related to Nile river runoff decreasing water-column oxygen, seawater surface density did not reach low enough values to lead to water-column stratification and associated sapropel formation. This is further supported by Fig. 7, indicating that this ‘missing sapropel’ interval does not enter the threshold temperature and salinity values of the ‘warm & fresh’ quadrant, required to slow down the overturning circulation enough for seafloor anoxia to develop.

During glacial stage 8 and at the end of isotopic stage 9 (going from interglacial to glacial), we observe ‘missing sapropels’ intervals at precession minima without sapropels between S9 and S10, characterized by high $\delta^{18}\text{O}$ values and low Ti/Al, in line with short episodes of enhanced Nile river runoff (Fig. 6). Yet as this is an older part of the record, the signal seems subdued due to diagenetic alteration and/or recrystallization. Evidence for higher nutrient inputs comes also from higher sedimentary Ba/Al, indicating enhanced productivity. Hence, continued mixing and/or convective turnover due to the colder temperatures may have counteracted higher Nile river input in these intervals as well, preventing sapropel formation.

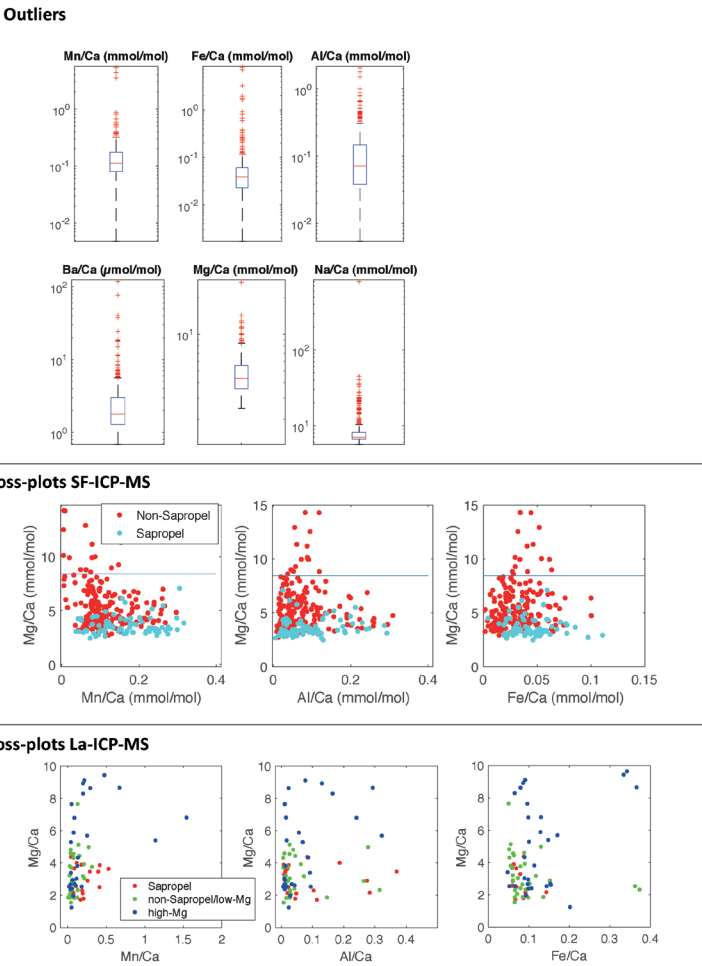
5.5 Conclusions

Patterns in foraminiferal Ba/Ca and Mn/Ca show variability related to surface water freshening in conjunction with sapropels, which inversely correlate to residual $\delta^{18}\text{O}$ and appear to reflect Nile-river runoff. Foraminiferal Na/Ca is not a suitable recorder of salinity down-core in the Mediterranean sedimentary record, most likely related to variable preservation of high Na parts of the foraminiferal shells. Foraminiferal $\Delta\text{Mg}/\text{Ca}$ appears a candidate for salinity estimates in a semi-enclosed basin such as the eastern Mediterranean due to the very large changes in salinity. Foraminiferal $\Delta\text{Mg}/\text{Ca}$ shows changes in salinity related to both seawater freshening during precession minima and hyper-saline phases during precession maxima. The combined proxy records show that orbital forcing also paced the eastern Mediterranean hydrology outside sapropel periods, when a threshold to sapropel conditions was not met, yet Nile water runoff still increased. Possibly cold temperatures prevented the build up of low density surface waters during these intervals.

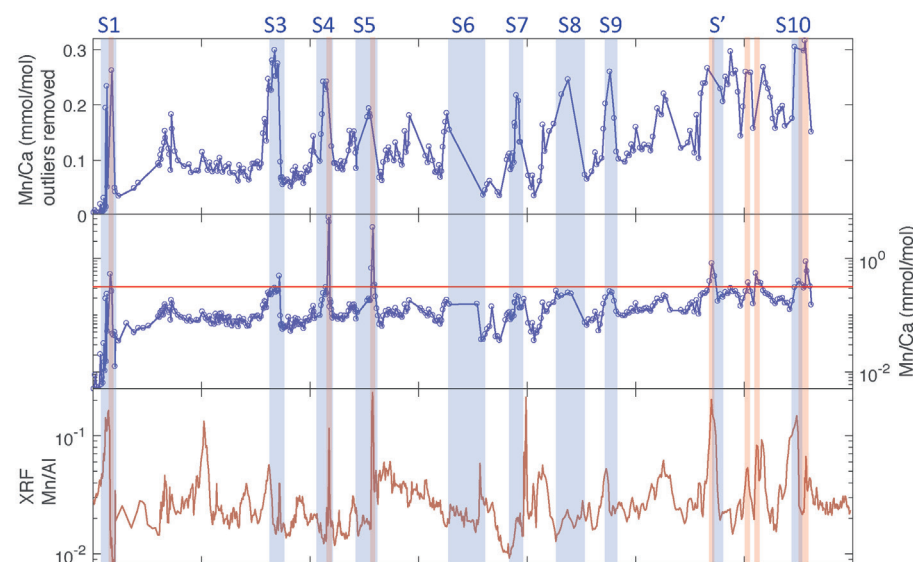
Acknowledgements

We thank Piet van Gaever and Wim Boer for analytical assistance. We are grateful to the captain and crew on board of RV Pelagia cruise 64PE406. This work was carried out under the program of the Netherlands Earth System Science Centre (NESSC), financially supported by the Ministry of Education, Culture and Science (OCW) (grant no. 024.002.001)

Appendix

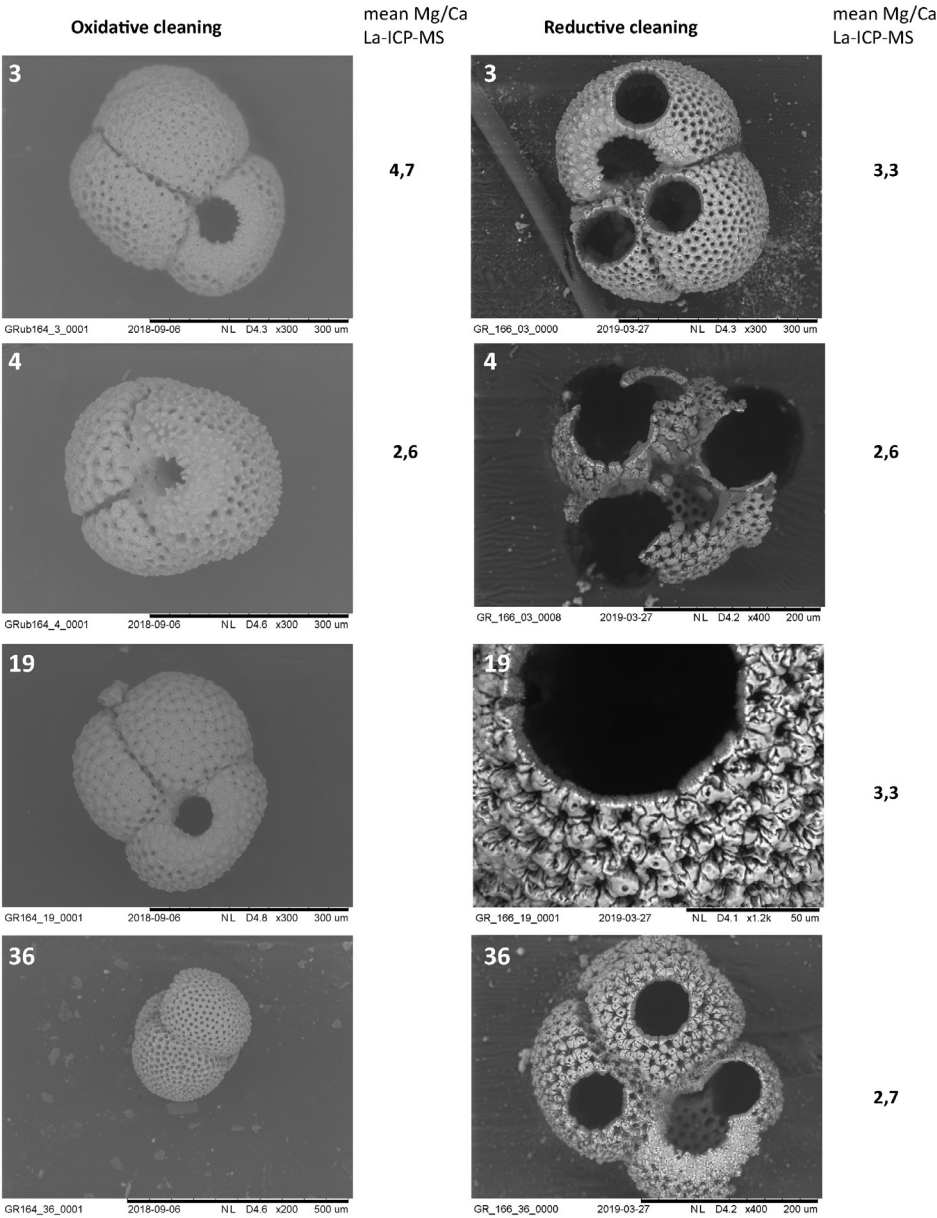


Appendix A1. Outliers and assesment of contaminant phases. Top panel. Boxplot showing distribution of SF-ICP-MS results for Mn/Ca, Fe/Ca, Al/Ca, Ba/Ca, Mg/Ca and Na/Ca. Outliers of the distributions are marked in red. Middle panel. SF-ICP-MS results for Mg/Ca against Mn/Ca, Al/Ca and Fe/Ca for sapropel (blue) and non-sapropel (red) samples, not showing significant correlations. The cut-off value for Mg/Ca, based on outlier analysis (see top figure), is indicated with a blue line. This shows that high Mg/Ca values are not related to high Al/Ca, Fe/Ca or Mn/Ca values. This excludes Mn-Fe coatings or clay contamination as cause for elevated Mg/Ca values. Bottom panel. La-ICP-MS values of individuals selected for SEM analysis (see Appendix B for SEM images and La-ICP-MS) profiles are comparable to the range obtained with Mg/Ca against Mn/Ca, Al/Ca and Fe/Ca for sapropel (red) and non-sapropel (green) samples, which do not show significant correlations either. This also confirms that high Mg/Ca values are not related to high Al/Ca, Fe/Ca or Mn/Ca values, excluding Mn-Fe coatings or clay contamination as cause for elevated Mg/Ca values.

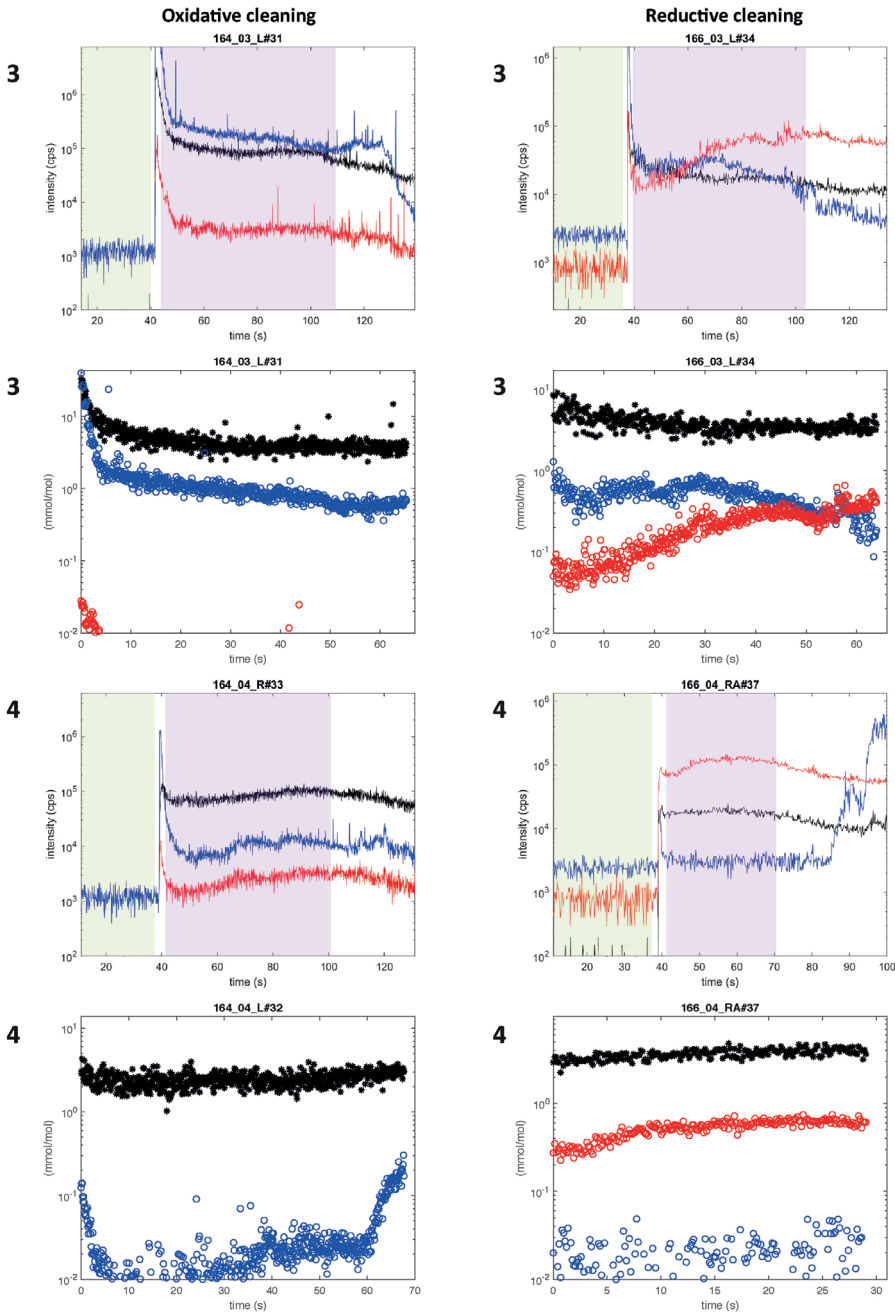


Appendix A2. Comparison of foraminiferal Mn/Ca, with the top figure showing the record without outliers and the middle figure showing the record without outliers removed (in red the Mn/Ca cut-off value, based on outlier analysis, see A1) and sediment Mn/Al, obtained with XRF in brown. Overall, there is no trend (and no significant correlation) between foraminiferal Mn/Ca and sediment Mn/Al, except that outliers in foraminiferal Mn/Ca are related to high Mn/Al peaks, often prior to the onset of sapropel, indicating a Mn-front. Foraminiferal Mn/Ca outliers are therefore removed for further analysis.

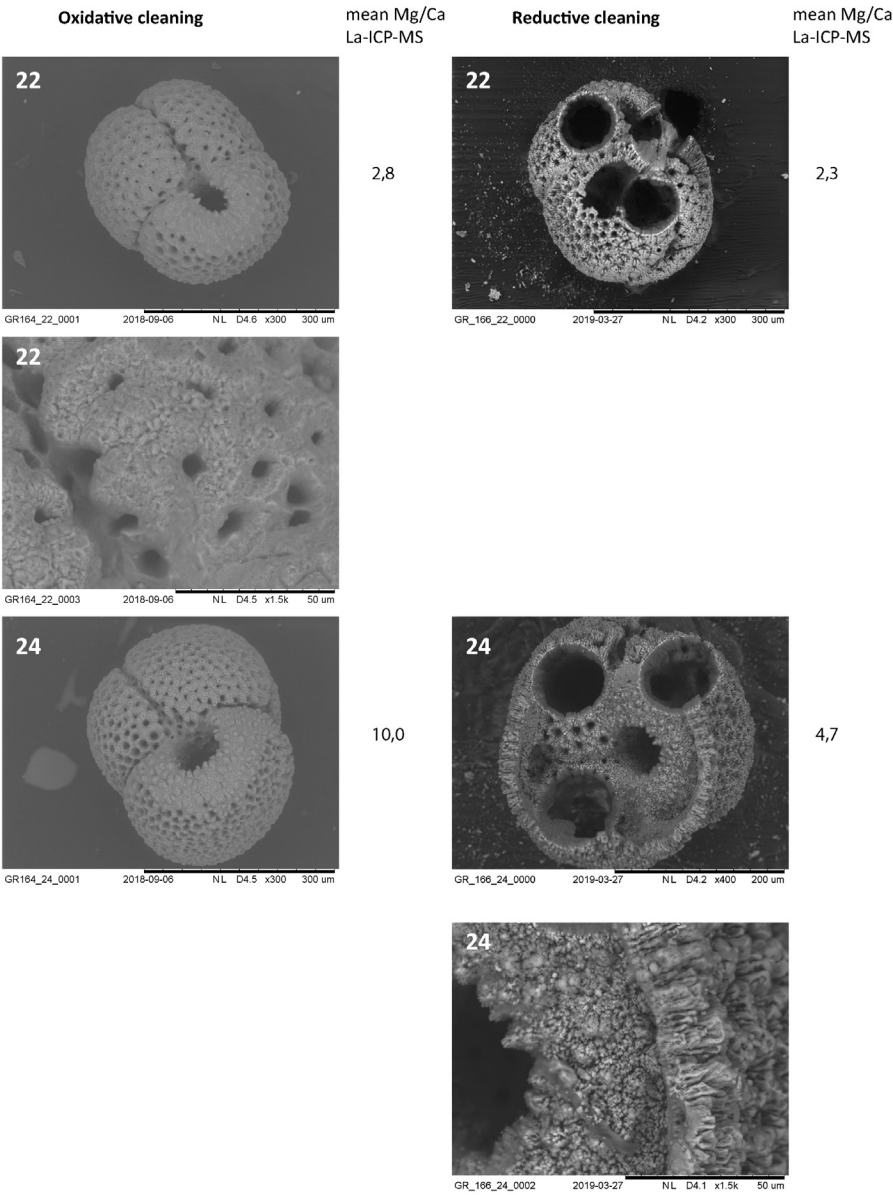
Appendix B. Examples of SEM images for selected specimens (specimen ID indicated in white letter), after an oxidative cleaning step and after a reductive cleaning step, showing no clear signs of inorganic overgrowths, for sapropel (B1), non-sapropel (B3) and non-sapropel high-Mg (B4, B5) samples. La-ICP-MS profiles of the same specimens (specimen ID indicated left of profile) are also shown, for sapropel (B2), non-sapropel (B4) and non-sapropel high-Mg (B7) samples. After the oxidative cleaning step SEM images of some specimens still show signs of coccolithophores encrusted in the shell wall, and high-Mg peaks are coinciding with high Al/Ca and Mn/Ca peaks, indicating clay contamination. In the SF-ICP-MS analysis Al/Ca is mostly below the threshold for clay contamination or otherwise removed, yet still Mg/Ca is high. After the reductive cleaning step, especially the high-Mg specimens are heavily corroded. This may be enhanced by applying the same volumes of reagents on individuals instead of a batch of ~20 specimens. However, the high-Mg peaks have been successfully removed. The first few seconds of the ablation-profiles often show high El/Ca peaks, which is an artifact of the Laser-ablation process, hence these peaks are usually omitted in the integration of the profile, see e.g. Steinhardt et al. (2015). Still, Mg/Ca in the high-Mg interval specimens appears to be elevated throughout the whole shell wall (~5-9 mmol/mol). This indicates that Mg/Ca in these specimens is higher than usual 'open ocean' foraminiferal Mg/Ca, yet not related to crusts of inorganic high-Mg overgrowths, which would yield values around ~100 mmol/mol.



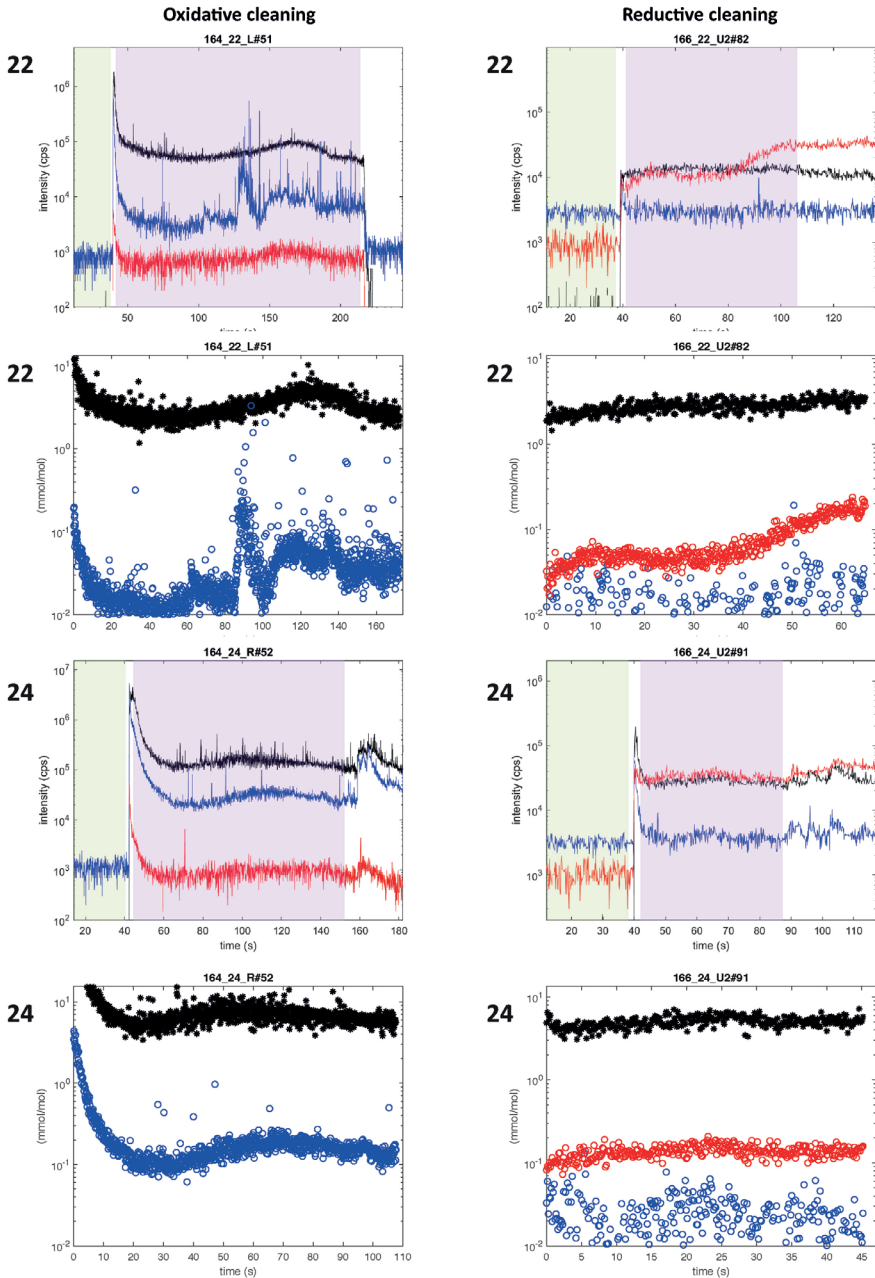
Appendix B1. Sapropel specimens



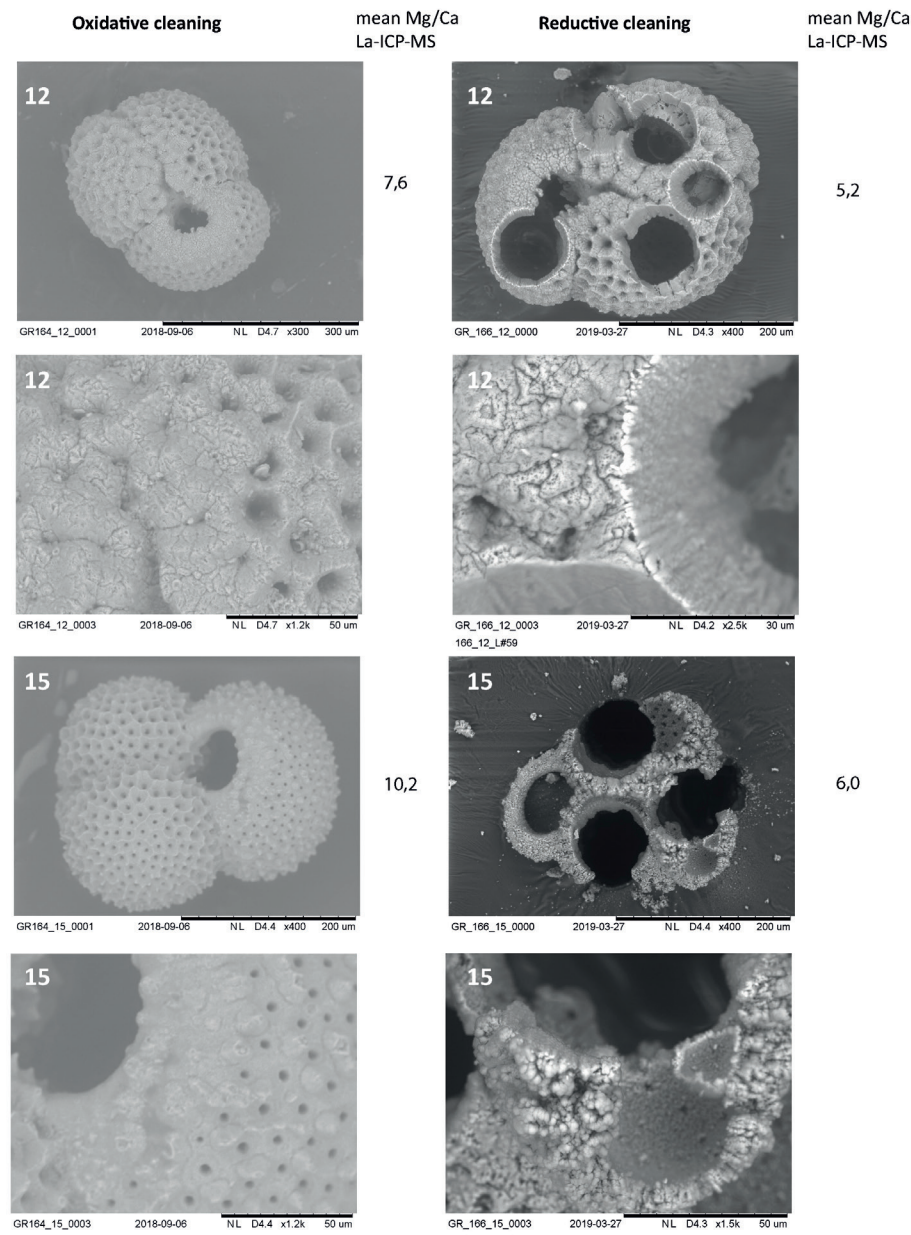
Appendix B2. La-ICP-MS profiles and ratios Sapropel specimens



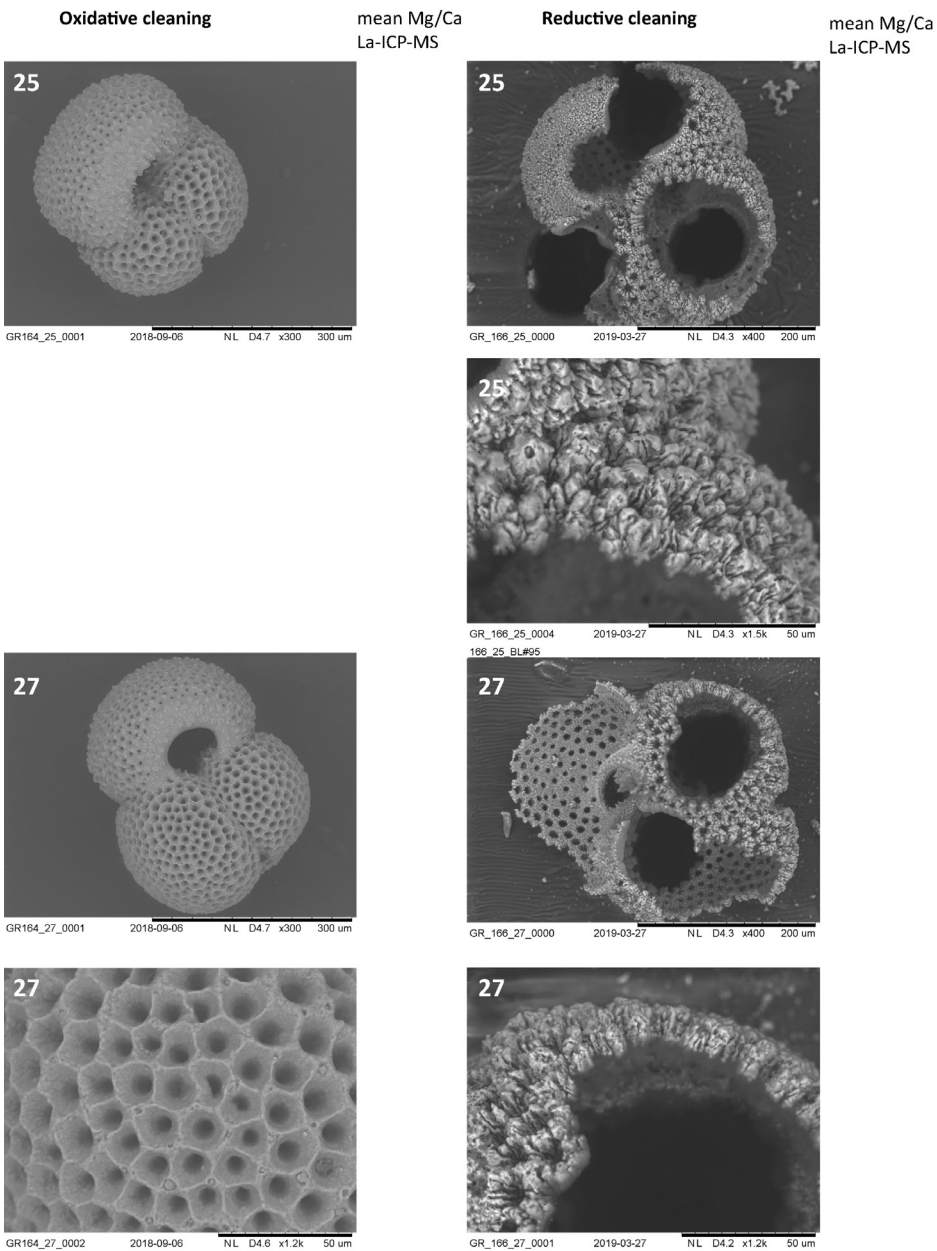
Appendix B3. Non-Sapropel specimens



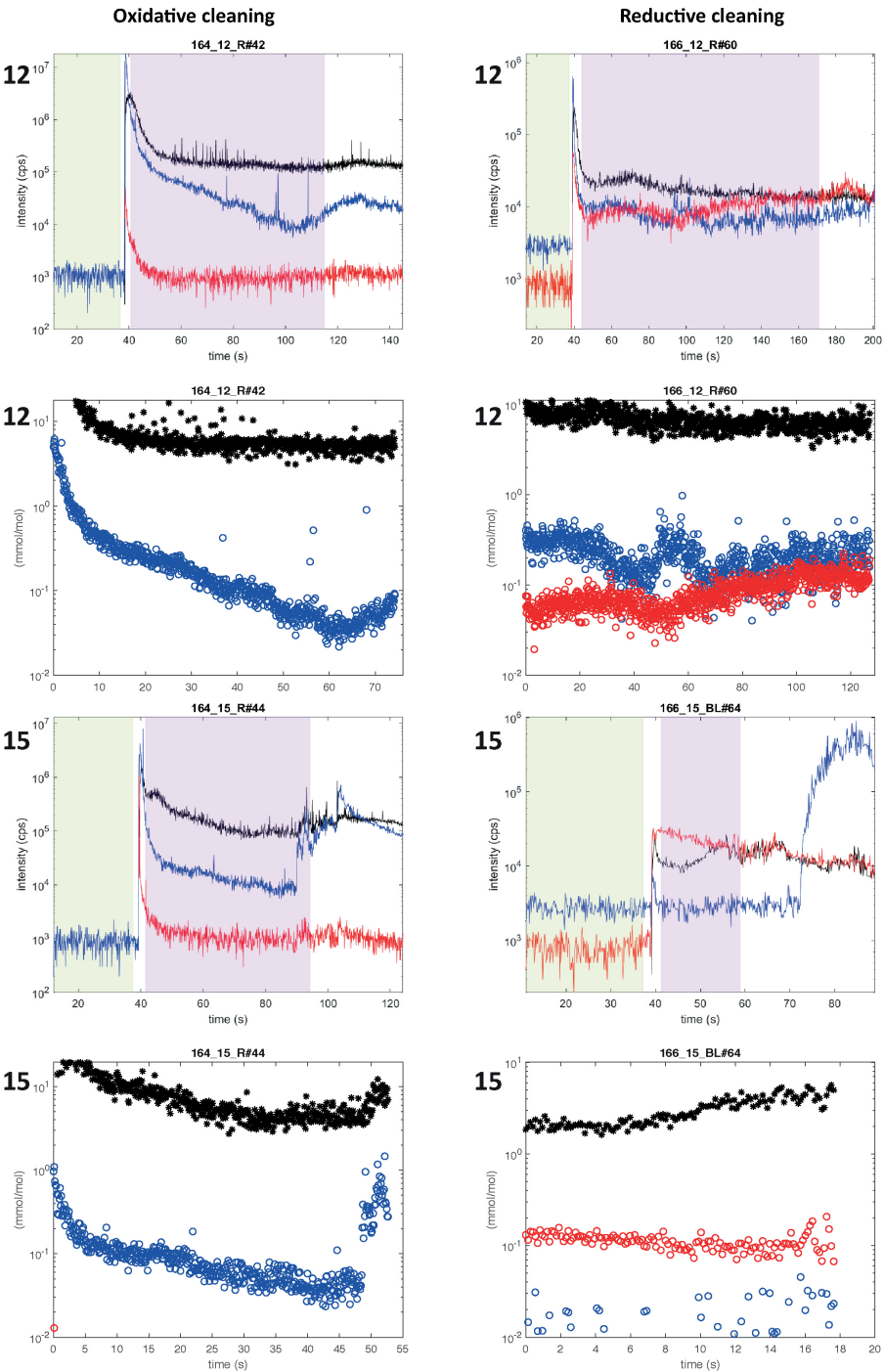
Appendix B4. La-ICP-MS profiles and ratios non-Sapropel specimens



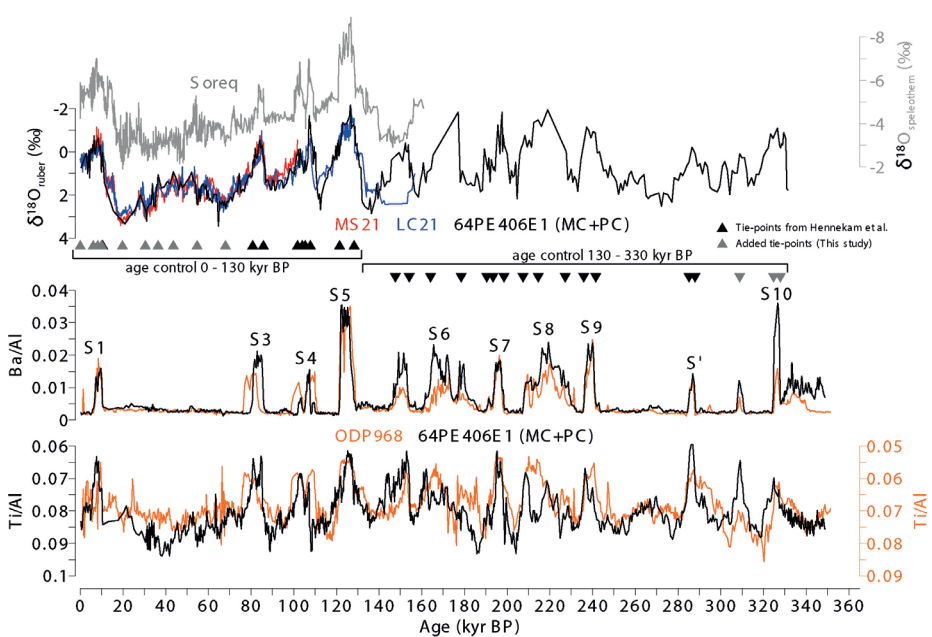
Appendix B5. Non-Sapropel high-Mg specimens



Appendix B6. Non-Sapropel high-Mg specimens



Appendix B7. La-ICP-MS profiles and ratios non-Sapropel high-Mg specimens



Appendix C. Age model and tie-points. Tuning of core 64PE406E1 to data from nearby cores MS21, LC21, and ODP968. Tie-points based on common variability in $\delta^{18}\text{O}$ and Ba/Al in our core 64PE406E1 and cores MS21 and LC21 are indicated by upward pointing triangles (0-130 kyr BP). The downward pointing triangles (>130 kyr BP) indicate the tie points between 64PE406E1 and ODP968, based on comparison of the Ba/Al records. The tie points previously used for the age model (Hennekam et al., In Review) are indicated with black triangles, while the grey triangles indicate the tie points that were added in this study. The common variability in the Ti/Al records of 64PE406E1 and ODP968 are used to verify the age model based on the sapropel boundaries.



Chapter 6

A biomineralization model explaining element incorporation and variability therein between foraminiferal species, specimens and within the shell wall

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6.1 Introduction

Trace metal incorporation in foraminiferal calcite is one of the most used proxies in paleoceanography, yet relationships to the environment are mostly determined empirically. The paleoceanographic value of foraminiferal shell chemistry would be greatly improved by a better understanding of their biomineralization pathways. More specifically, identification of the biochemical processes involved in calcification is needed to explain i) the difference between inorganic- and foraminiferal calcites ii) the (in) dependency of element incorporation on environmental parameters and iii) differences in calcite chemistry within (i.e. element banding) and between specimens and species. Over the last few decades a number of conceptual and numerical biomineralization models explaining element incorporation in foraminiferal calcite have been developed (Fig. 1). Although these models all explain certain aspects of foraminiferal geochemistry, accounting for variability in the involved parameters is challenged by the complexity of foraminiferal biomineralization. However, an adequate biomineralization model should be able to explain differences in patterns in element simultaneously across scales, such as both banding and differences between species.

Abstract

Foraminifera precipitate their shells under tight biological control. Various proposed biomineralization models for element incorporation aim at explaining offsets between foraminiferal and inorganically precipitated calcites and differences between foraminiferal species. Here we combine fractionation from a limited reservoir, such as the Rayleigh fractionation model as described by Elderfield et al. (1996), with the trans-membrane-transport model. This new model is set up as to explain variability in element incorporation at three levels: within the shell wall, between specimens and between species. The model is based on calcite precipitation from a confined biomineralization space, which is initially filled with seawater. Inwards Ca^{2+} -transport during calcification and incorporation of elements present in seawater decreases the El/Ca ratio in the reservoir, and hence the El/Ca ratio of the shell wall. Variability in the volume of the reservoir, shell thickness and inorganic partitioning coefficients may explain observed variability in element incorporation between species, specimens and within the shell wall observed in two species of benthic foraminifera: *Ammonia tepida* and *Amphistegina lessonii*.

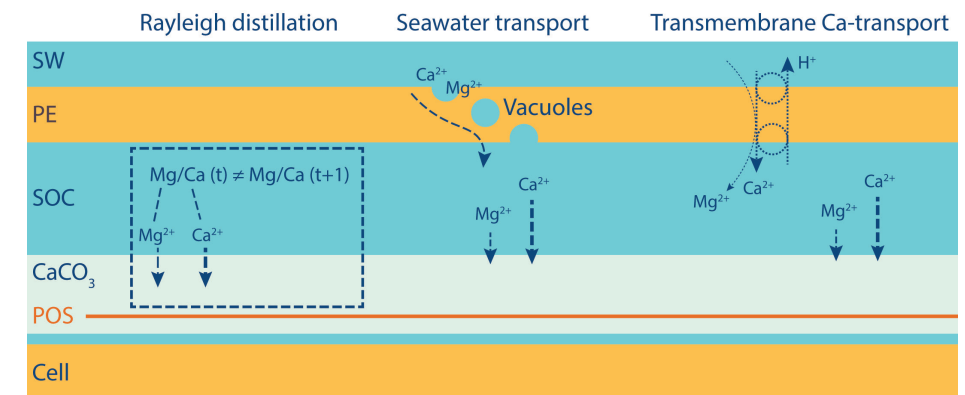


Figure 1. Three previously proposed models for foraminiferal biomineralization. Each of these processes is here taking place simultaneously within the site of calcification (SOC) that is separated from the surrounding seawater (SW) by the protective envelope (PE). The CaCO_3 that forms the new chamber wall contains the primary organic sheet (POS) on which the first layer of calcite precipitates. Left: Rayleigh fractionation model of calcification as suggested by Elderfield et al. (1996) in which CaCO_3 precipitates from a reservoir (dashed square) so that the Mg/Ca changes over time. Middle: seawater transport by vacuoles delivering calcium- and other ions to the SOC (Bentov et al., 2009; Erez, 2003) in the original model, Mg^{2+} is removed from the seawater-containing vacuoles. Right: selective transmembrane Ca-transport by exchanging Ca^{2+} for H^+ (Nehrke et al., 2013; Toyofuku et al., 2017). This type of calcium transport assumes an accidental transport of other ions (e.g. Mg^{2+}) from the surrounding seawater into the SOC.

A pioneering model of element partitioning in foraminiferal calcification based on Rayleigh distillation from an enclosed biomineralization reservoir, initially filled with a seawater-like solution was proposed by Elderfield et al., (1996). With ongoing precipitation, Ca^{2+} within the reservoir is depleted, while other elements become depleted or enriched (relative to calcium), depending on their inorganic partitioning coefficients. Elderfield et al. (1996) suggest that the reservoir may be replenished with seawater in between the formation of consecutive chambers and that Ca and carbon may be provided from internal pools. Furthermore, the authors suggest that variability in the reservoir volume may explain differences in elemental composition between species.

Based on this model, if all incorporated Ca^{2+} would be precipitated from a closed reservoir, its' size would have a profound impact on element signatures. In fact, even for the precipitation of a single chamber, the amount of Ca^{2+} in a reasonably sized reservoir would not suffice, as a seawater volume of approximately 75 times the volume of a foraminiferal specimen would be required to provide sufficient Ca^{2+} for a single chamber (de Nooijer et al., 2009b). This renders flushing of the calcification reservoir in between the formation of subsequent chambers an unlikely mechanism to collect the Ca^{2+} required. Therefore, Bentov and Erez (2006) suggested that seawater is vacuolized (either before or during chamber formation), based on observations on intermediate-Mg species *Amphistegina lessonii*, and that Mg is exported outwards to reduce the Mg content of the calcite. In this model element banding in Mg is explained by precipitation of a high and a low Mg phase. In low-Mg species, Ca^{2+} has been shown to be taken up directly from the culture medium during calcite precipitation (Nehrke et al., 2013, Chapter 4), which also renders internal pools an unlikely source for Ca^{2+} , at least for these species. Instead, these results suggest that Ca^{2+} is supplied during calcification, which provided the basis for the trans-membrane ion transport model (Nehrke et al., 2013). This model assumes that there is a large flux of calcium ions from the surrounding seawater into the site of calcification (SOC) by means of Ca^{2+} -transporters, comparable to models for coral-calcification (Cai et al., 2016; Sinclair et al., 2006). A difference in Mg between foraminiferal species in this model is explained by different amounts of 'passive transport' of seawater, e.g. by seawater vacuolization (Nehrke et al., 2013).

Here we combine aspects of both models: precipitation from a finite biomineralization space initially filled with seawater (Elderfield et al., 1996) and Ca^{2+} -transport and seawater leakage (Nehrke et al. 2013). By specifying different, variable volumes for the SOC and shell wall thicknesses, we can test whether element geochemistry can be explained using observed (or reasonable) parameters, including seawater element concentrations and inorganic partition coefficients. Furthermore, we test if this model

may explain observed variability in El/Ca between species, specimens and within the shell wall. The model results are therefore compared to geochemical data for *Ammonia tepida* (precipitating low-Mg/Ca calcite) and *Amphistegina lessonii* (producing calcite with an intermediate-Mg/Ca), presented in this thesis and elsewhere: correlated element banding within the shell wall, (e.g. Geerken et al., 2019; Spero et al., 2015), correlated elements between specimens (Geerken et al., 2018) and between species (Van Dijk et al., 2017b). Eventually, this model may be extended and used to mechanistically explain the effect of changes in seawater variables (e.g. salinity and temperature) on element incorporation in foraminiferal calcite.

6.2 Model outline and supporting observations

Our model is based on the enclosure of a small volume of seawater at the onset of chamber formation, which remains separated from seawater during calcite precipitation, while Ca^{2+} -ions are transported inwards (Fig. 2). This is based on observations of the SOC being shielded by a protective envelope during chamber formation (Bé et al., 1979). Enclosure of the SOC from seawater is further supported by pH being increased inside (de Nooijer et al., 2009; Toyofuku et al., 2017), while outside of the membrane pH decreases during calcification (Glas et al., 2012; Toyofuku et al., 2017, chapter 4). This pH-gradient is furthermore suggested to be linked to transport of Ca^{2+} and carbon ions into the SOC, driven by coupled proton- Ca^{2+} pumping (Toyofuku et al., 2017). Within the SOC, calcite precipitates lamarily on top of the primary organic sheet (POS), which is supported by focused ion beam observations (Nagai et al., 2017) and labeled precipitation rate experiments (chapter 4) capturing chamber formation. Elements are incorporated into the calcite according to the SOC's El/Ca and the inorganic partition coefficients, as established in inorganic precipitation experiments (e.g. Rimstidt et al., 1998; Tesoriero and Pankow, 1996). Since partition coefficients depend on the CaCO_3 precipitation rate, estimated coefficients are used for high rates when available.

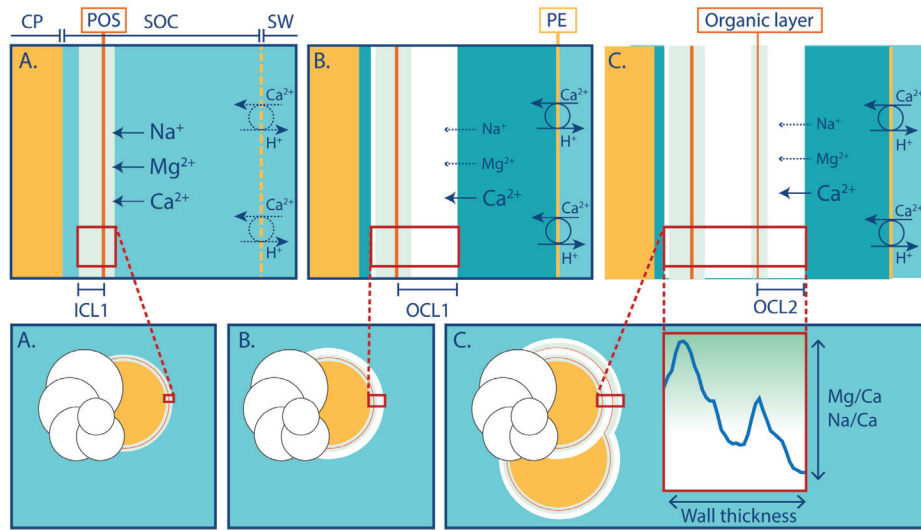


Figure 2. A model for foraminiferal biomineralization explaining banding of Na and Mg within perforate chamber walls. A. Initial enclosure of seawater captures all ions present in seawater at the site of calcification (SOC), after which the protective envelope (PE, orange) seals the SOC from the surrounding medium. Once the proton gradient is being built up by active H^+/Ca^{2+} exchange, a first layer of calcite is formed onto the POS due to the increased saturation state at the SOC (inner calcite layer (ICL), green). The Mg and Na present in the initial volume of seawater are precipitated with this first layer of calcite, resulting in a band of high Mg/Ca and Na/Ca close to the POS. B. As calcification commences, the concentrations of cations other than Ca^{2+} decrease as they are discriminated against during transmembrane pumping of Ca^{2+} , resulting in calcite layers with relatively low Mg and Na concentrations (outer calcite layer (OCL), white). The transmembrane Ca^{2+} transporter may not discriminate against other (divalent) cations to the same extent, so that some elements (e.g. Sr^{2+}) remain present in ratios to Ca similar to seawater (see chapter 4). C. The sequence is repeated with the precipitation of subsequent chambers, when a (thinner) layer of primary and subsequently, secondary calcite envelopes previous chambers, creating the characteristic lamella of high and low Mg/Ca and Na/Ca.

According to the model presented here, summarized in Fig. 2, a foraminifer's average El/Ca_{cc} depends on i) the volume of enclosed seawater, ii) the eventual wall thickness, iii) the element concentration in seawater and, iv) inorganic partition coefficients, of which the latter depends on precipitation rate and environmental parameters. The main difference with the Rayleigh distillation model of Elderfield et al. (1996) is that we add (variable) Ca^{2+} -transport, basically counter-acting the effect of Rayleigh distillation on element incorporation. Furthermore, and here the model differs from Nehrke et al. (2013), we include a volume of seawater at the onset of calcification, with a specified SOC-volume and account for wall thickness. The model therefore allows assessing if average El/Ca values as well as element banding within the shell wall, can be explained for different foraminiferal species, with parameterization falling within observed values (if available).

6.3 Numerical application

In our model output, El/Ca ratios across the shell wall depend on i) the element's concentration in seawater, ii) the inorganic partition coefficients and iii) the thickness of the SOC and shell wall. Consumption of cations (other than calcium) from the SOC and incorporation into the shell wall is described by an exponential function.

A range of inorganic partition coefficients (D) for Mg, Na and S are obtained from experiments in which calcite is precipitated from fluids resembling seawater to maximize the resemblance to $CaCO_3$ precipitation from the SOC (D_{Mg} : Burton and Walter, 1991, D_{Na} : Mezger et al., in prep, D_{SO_4} : Busenberg and Plummer, 1985). The volume of the SOC, which is measured as a thickness relative to the test wall, has been observed to be about $1\mu m$ extending with the growing calcite front for *A. tepida* (Nagai et al., 2018). This is similar to values observed for low-Mg/Ca calcite benthic (Angell, 1979) and planktonic (Bé et al., 1979) species.

We here first assume that the calcium concentration ($[Ca^{2+}]$) in the SOC remains constant at seawater concentration, which implies that the consumption of Ca^{2+} by calcite precipitation is compensated by an equal transport flux of Ca^{2+} into the SOC. Since $[El]$ is decreasing, and $[Ca^{2+}]$ remains constant, the El/Ca ratio in the SOC is decreasing at each precipitation step, hence the El/Ca ratio of the calcite decreases as the lamella grows. Below our model is explained numerically, with the following parameters defined:

- w_{SOC} : the width of the SOC layer (Fig. 3)
- W : the width of the precipitated calcite (Fig. 3)
- A : the area of the entire calcite sheet
- V : the volume of the SOC, which by the above definitions equals $A * w_{SOC}$

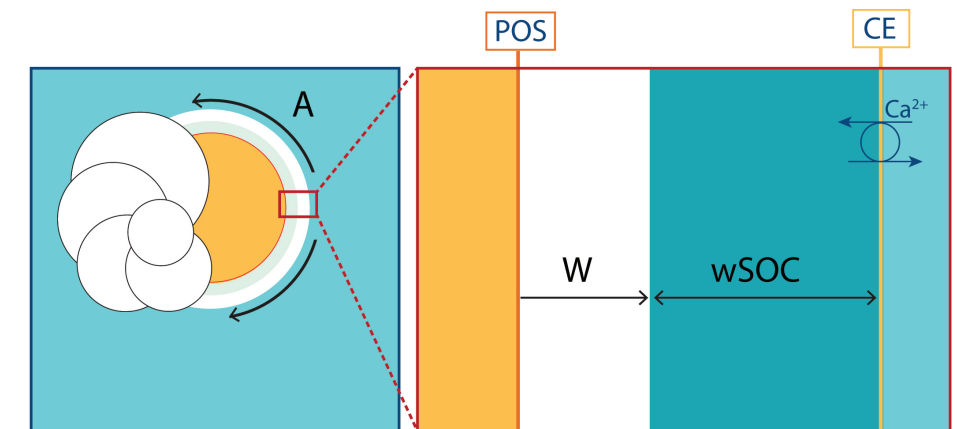


Figure 3. Basic parameters of the model.

We assume that at each moment in time, the concentration of element El relative to calcium in the precipitating calcite is linearly related to its relative concentration in the SOC, according to

$$\left(\frac{[El]}{[Ca]}\right)_{cc} = D_{El} \frac{[El]}{[Ca]} \quad (\text{Eq. 1})$$

with El representing the elements of interest, Na, Mg and S. D_{El} is the element-specific inorganic partition coefficient. We will use the convention that elements in brackets represent their concentrations in the SOC in mol/liter (as in $[Ca]$), while elements without brackets refer to their total amount in the SOC in mol (as in Ca).

Assuming that the foraminifera precipitate a sheet of calcite with an infinitesimal width dW , we calculate the amount of Ca added to the shell wall from the SOC accordingly:

$$Ca_{precip} = M_{Ca} A dW \quad (\text{Eq. 2})$$

with M_{Ca} the molarity of calcium in calcite (27.1 mol/l)

Combining Eq.1 and 2, we find that in the precipitation process, the amount of elements precipitated other than calcium, El_{precip} in the calcite equals:

$$El_{precip} = \left(\frac{[El]}{[Ca]}\right)_{cc} * Ca_{precip} = D_{El} \left(\frac{[El]}{[Ca]}\right)_{SOC} * Ca_{precip} = D_{El} \left(\frac{[El]}{[Ca]}\right)_{SOC} * M_{Ca} A dW$$

Given the constant precipitation rate with ongoing chamber formation (Chapter 4) we here assume that the Ca precipitated from the SOC is fully replenished with new Ca added from seawater, such that $[Ca]$ in the SOC remains constant. Consequently ongoing precipitation will produce an infinitesimal change $d[El]$ in the element concentration $[El]$ in the SOC, according to:

$$d[El] = \frac{-El_{precip} + El}{V} = \frac{\left(D_{El} \frac{[El]}{[Ca]}\right) M_{Ca} A dW}{A w_{SOC}}$$

which we may rearrange to:

$$\frac{d[El]}{dW} = (-B [El])/w_{SOC} \quad (\text{Eq. 3})$$

with

$$B = \frac{M_{Ca} D_{El}}{[Ca]}$$

The differential equation then has a simple exponential solution:

$$[El] = [El_0] e^{-B W/w_{SOC}} \quad (\text{Eq. 4})$$

where $[El_0]$ is the starting concentration of an element E in the SOC. Using Eq. 1 we may rewrite this into an expression that relates the element-to-calcium ratio in the calcite to the thickness of the calcite layer W (i.e. position with respect to POS):

$$\left(\frac{[El]}{[Ca]}\right)_{cc} = D_{El} \frac{[El_0]}{[Ca]} e^{-B W/w_{SOC}} \quad (\text{Eq. 5})$$

For later comparison to observations we also calculate the average element-to-calcite ratio over the full width of a lamelle $D_{lamelle}$, given by:

$$avg \left(\frac{[El]}{[Ca]}\right)_{cc} = \frac{\left(\int_0^{D_{lamelle}} D_{El} \frac{[El_0]}{[Ca]} e^{-B \frac{W}{w_{SOC}}} dW\right)}{D_{lamelle}} = \frac{[El_0]}{[Ca]} \frac{D_{El}}{B w_{lamelle}/w_{SOC}} (1 - e^{-B w_{lamelle}/w_{SOC}}) \quad (\text{Eq. 6})$$

Parametrization

Inorganic partition coefficients D_{El} as used for output presented in Figs. 4 and 5, in Fig. 6 D_{El} is indicated in plot titles, based on ranges for different temperatures.

$D_{Mg} = 1.5e-2$ (Burton and Walter, 1991)

$D_{Na} = 2e-3$ (Mezger et al., in prep)

$D_S = 50e-5$ (Busenberg and Plummer, 1985)

Element concentration in seawater, $[El_0]$, at salinity 35 (values from culture media in Geerken et al., 2018)

Na= 4.69e-1 mol/L

Mg= 5.30e-2 mol/L

Sr= 9.10e-4 mol/L

Calcium concentration in seawater $[Ca]$, at salinity 35

$Ca_{sw} = 1.03e-2$ mol/L

When assuming variable Ca^{2+} -transport

$$\text{Ca}_{\text{sw-beginning}} = 1.03 \times 10^{-2} \text{ mol/L}$$

$$\text{Ca}_{\text{sw-end}} = 3.03 \times 10^{-2} \text{ mol/L}$$

6.4 Model results and discussion

Rather than aiming at a perfect reproduction of observed El/Ca ratios and element banding in foraminiferal calcite, we here aim to assess how differences in the defined parameters (SOC volume, El/Ca in the SOC, wall thickness and inorganic partition coefficients) would affect average shell El/Ca values and element banding patterns within the shell wall. Outcomes are subsequently compared to observations for *A. lessonii* and *A. tepida* (Chapters 3 and 4).

6.4.1 SOC volume and wall thickness

Focussed ion beam observations on *A. tepida* show that the SOC thickness (i.e. the distance between the calcite growth front and the protective envelope; Fig. 3) is approximately 1 μm at the onset of calcification (Nagai et al., 2018). This thickness appears to remain constant during thickening of the chamber wall (Nagai et al., 2018), rather than a SOC volume that decreases over time with the SOC being filled up with the thickening shell during calcification. For *A. lessonii*, a larger species than *A. tepida* (see Chapter 2), the SOC may well be somewhat larger, as we assume here, or may additionally be resupplied with seawater during chamber formation by seawater vacuoles (Bentov et al., 2009). To assess the effect of SOC volume on element incorporation we numerically investigate the effect of a range of probable SOC thicknesses, ranging from 0.1 to 10 μm , on element incorporation (Fig. 4).

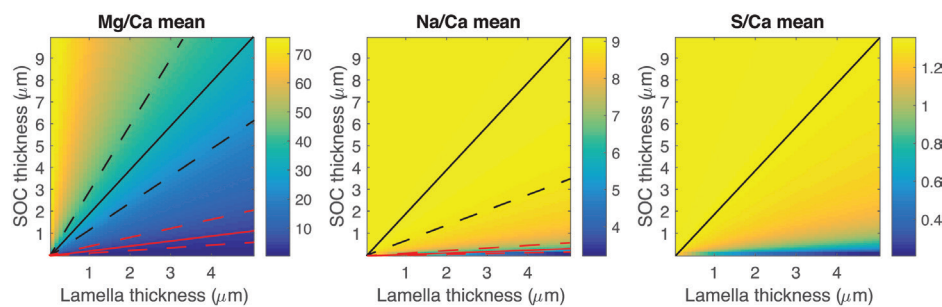


Figure 4. Model results showing the average Mg/Ca, Na/Ca and Sr/Ca as a function of thickness of the SOC and the thickness of the precipitated chamber wall. Indicated are the approximate average El/Ca for two species (results from chapter 2 of this thesis; Geerken et al., 2018).

Inherently, average El/Ca increases with increasing SOC thickness, as in total, more El is available within a larger SOC (Fig. 4). An increase in wall thickness decreases the average El/Ca, as more ‘element-depleted’ calcite is precipitated from the limited El pool (Fig. 4). Wall thickness depends on live-stage, yet on average, wall thickness is taken here as ~3-5 μm for both species (Geerken et al., 2019, chapter 4). Furthermore, Fig. 4 shows that especially Mg/Ca is sensitive to changes in SOC and wall thickness, because of the relatively high D_{Mg} . The difference in El/Ca between *A. lessonii* and *A. tepida*, which is like between most species especially large for Mg/Ca (Van Dijk et al., 2017b), can hence simply be explained by a change in SOC thickness. For example, a wall thickness of 5 μm and a SOC thickness of 1 μm would yield Mg/Ca values of 4 mmol/mol (Fig. 5), matching observations for wall thickness, SOC thickness and average Mg/Ca values for *A. tepida* (~3±1 SD mmol/mol; Geerken et al., 2018). With parameters defined accordingly, values for Na/Ca of 8 mmol/mol also fall within the order of magnitude of observed values for *A. tepida* as well (~6±1 SD mmol/mol; Geerken et al., 2018). Inter-specimen variability observed for Mg/Ca (ranging from ~2-4 mmol/mol; Geerken et al., 2018) can be explained by differences in the SOC thickness from 0.5 to 1 μm to or wall thickness varying from 5 to 10 μm (Fig. 4). Although SOC thickness is not constrained by observations for *A. lessonii*, a SOC thickness of 10 μm for a wall thickness of 5 μm yields values (34 mmol/mol, Fig. 5) observed for *A. lessonii* at a salinity of 35 (~33±10 SD mmol/mol; Geerken et al., 2018). Observed inter-specimen variability in Mg/Ca, ranging from ~23 to 43 mmol/mol, can be explained by changes in the SOC volume from 5 to 15 μm or wall thickness varying between 3 and 7 μm (Fig. 4).

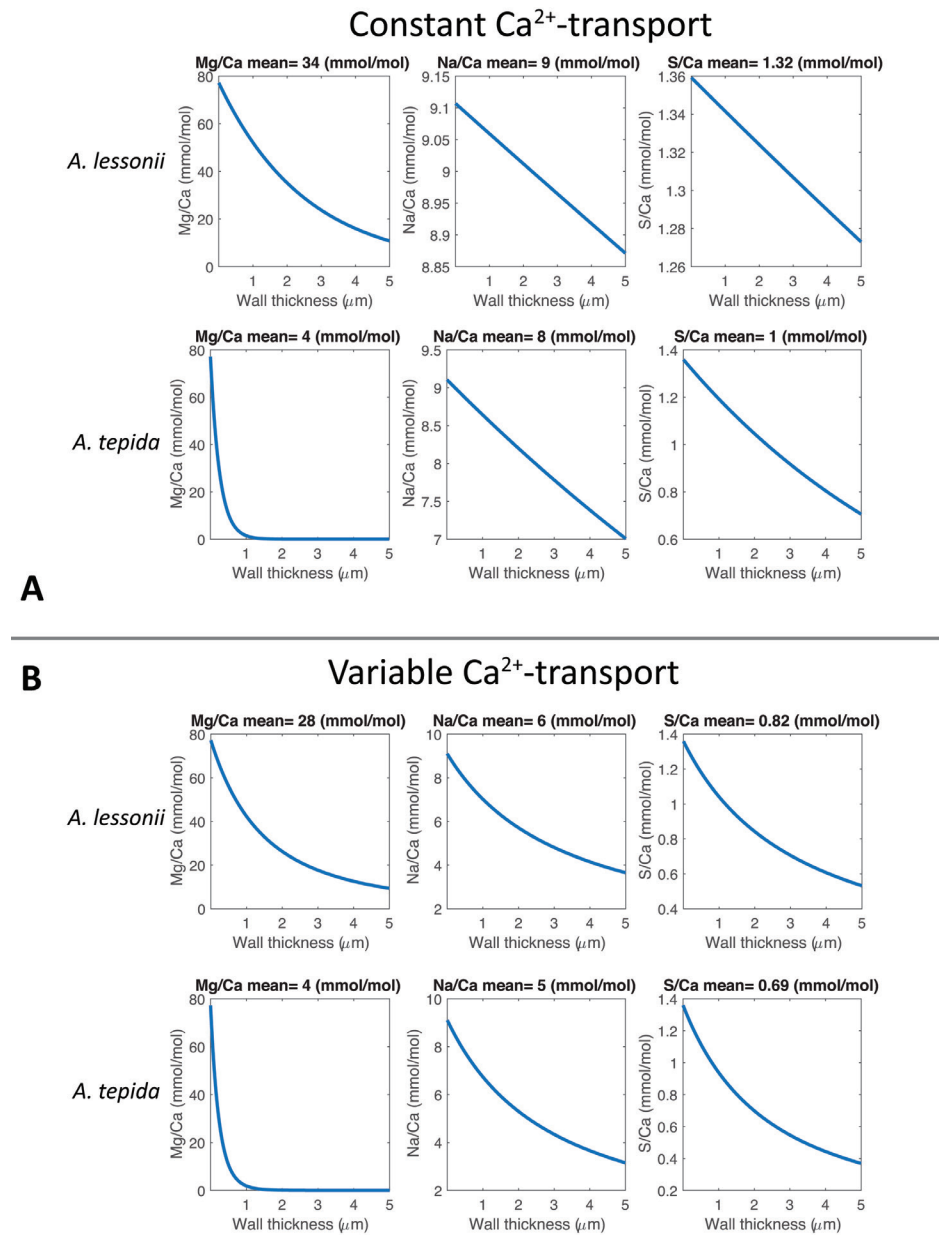


Figure 5. Comparison of modeled changes in El/Ca for a final SOC thickness of 5 μm (upper panels) with profiles from EPMA-measured profiles in those elements from cultured specimens of *A. tepida* and *A. lessonii* (lower panels).

Our model implies that the location element bands within the shell wall coincide with the first calcite precipitated on the primary organic sheet (POS), which is in line with nanoscale observations of the high-concentration element bands in benthic foraminifera (e.g. Geerken et al., 2019). Element banding in our model results from the change in El/Ca , initially at seawater concentrations, in the SOC over time, due to El being incorporated, while Ca is continuously supplied to the SOC. This furthermore explains the observed spatial co-occurrence of high-concentration bands for different elements (Geerken et al., 2019). However, the calculated relative amplitude of the banding pattern for Na and S is less than that observed, and variability in Na/Ca due to changes in SOC volume and wall thickness is lower than observed between the species, when using SOC volumes needed to explain Mg/Ca inter-specimen variability. This suggests there is another variable that needs to be taken into account.

6.4.2 Partition coefficients

Inorganic partitioning coefficients depend on calcite precipitation rate (Böhm et al., 2012; Busenberg and Plummer, 1985; Lorens, 1981; Mucci, 1986; Mucci and Morse, 1983; Tang et al., 2008) and environmental variables such as e.g. temperature and salinity (e.g. Burton and Walter, 1991; Kitano et al., 1975; Tesoriero and Pankow, 1996). Foraminiferal precipitation rates are comparable to the highest rates obtained in a limited number of inorganic precipitation experiments (Chapter 4). Accordingly, inorganic partition coefficients for calcite precipitated at such high rates have only been quantified for a few elements, such as for D_{Sr} (Böhm et al., 2012). The effect of temperature on partition coefficients has been quantified, albeit at lower precipitation rates, and we therefore here use D_{Mg} values obtained from inorganic precipitation experiments ranging from 5 to 45°C (Burton and Walter, 1991), to assess the thermodynamic effect of temperature on Mg/Ca (Fig. 6). D_{Na} may vary due to various factors, such as precipitation rate or salinity (Ishikawa and Ichikuni, 1984), and we estimated a range based on inorganic precipitation experiments from Mezger et al., in prep. D_{S} is based on a range obtained for different precipitation rates (Busenberg and Plummer, 1985). Altogether, El/Ca variability between species and specimens may hence also vary due to differences in D_{El} , for instance, a different precipitation rate between species, may explain different inter-specimen correlations between Na/Ca and Mg/Ca between *A. tepida* and *A. lessonii* (Geerken et al., 2018; 2019).

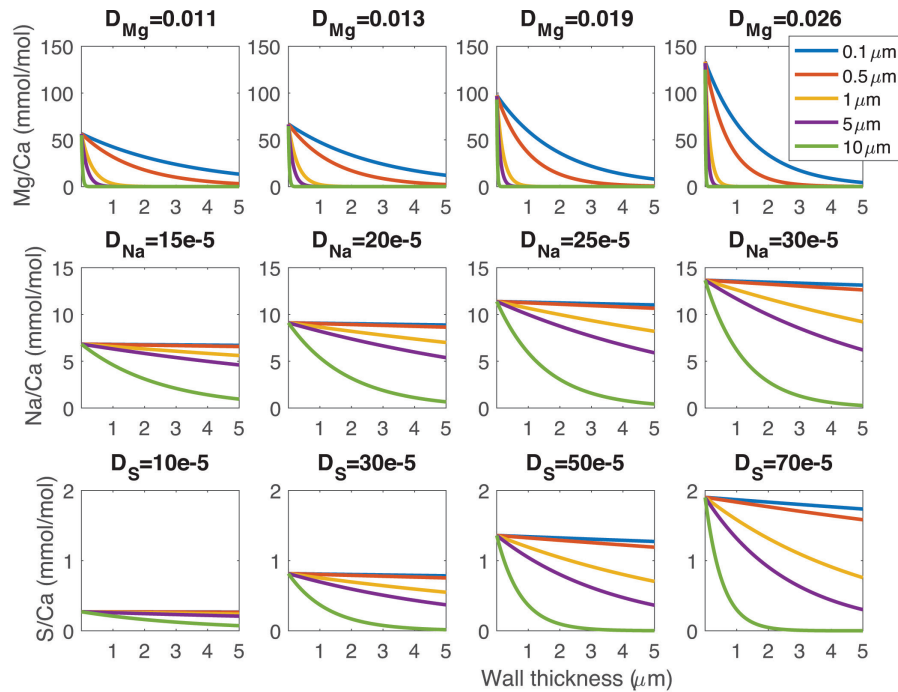


Figure 6. Modeled Mg/Ca (upper panels), Na/Ca (middle panels) and S/Ca (lower panels) as a function of SOC volume (indicated in legend) for wall thicknesses between 0 and 5 μm , the inorganic partitioning coefficient (D_{El}) as indicated in figure title. As D_{El} is essentially unknown in foraminiferal biomineralization and also likely to vary with environmental conditions, we here explore hypothetical variability in D_{El} values. Values for D_{Mg} are based on precipitation from inorganic calcite experiments for temperatures of 5, 27, 36 and 45 $^{\circ}\text{C}$ at log $p\text{CO}_2$ of -2 (from left to right; Burton and Walter, 1991). A range for D_{Na} is estimated based on inorganic calcite experiments of Mezger et al., in prep. A range for D_{S} is based on the range of D_{SO_4} for different growth rates found by Busenberg and Plummer, 1985.

Naturally, a higher D_{El} raises the El/Ca average, resulting especially in higher peak values and to a lesser extend, higher trough values, if the element is not depleted from the SOC before the end of chamber formation (Fig. 6). In our model, this is the case for Mg/Ca precipitated from a small SOC volume (e.g. for *A. tepida*; Fig. 5). A (proportional) increase in both peak and trough values within chamber wall profiles is in line with observations of element banding for increasing temperatures (Fehrenbacher et al., 2017; Geerken et al., 2019; Spero et al., 2015). For elements with a relatively high inorganic D_{El} (such as Mg when compared to Na) the amplitude in banding will be high. Hence, modelled Mg/Ca shows high peak values that become rapidly depleted with increasing wall thickness as the element is incorporated, especially for smaller SOC volumes (Fig. 6). Elements with a relatively low inorganic D_{El} and high seawater concentration, such as Na^+ , our model results in only gradually decreasing El/Ca levels with increasing shell

thickness, as these elements are barely incorporated into the calcite and hence remain high in the SOC. Only for very small SOC volumes ($\sim 0.1 \mu\text{m}$), these elements show more amplified banding. Such small SOC volumes are, however, not in line with observations and do not yield observed average Mg/Ca and Mg-banding patterns. Although Na/Ca banding has a lower amplitude than Mg/Ca banding, hence is more gradually declining, observations do show more pronounced differences between peak and trough values (Geerken et al., 2019). This suggests that another variable is important in explaining element banding and inters-specimen variability in El/Ca.

6.4.3 Variable Ca^{2+} -transport

Element incorporation as a function of wall thickness may additionally be affected by variability in Ca^{2+} -transport during chamber formation. The Ca^{2+} - H^+ pumping may rely on the production of protons by the inward-diffusing CO_2 (Toyofuku et al., 2017), so that the flux of Ca^{2+} may not (immediately) match the carbon-influx. This could result in a variable Ca^{2+} -influx over time and hence an additional factor affecting El/Ca in the SOC over time. Such a deviation from a constant flux is supported by the observed pH-fluctuations outside the SOC, suggested to reflect Ca^{2+} -proton pumping (Toyofuku et al., 2017), which decreases at the onset of calcite precipitation, subsequently reaching an optimum before increases again towards the end of chamber formation (Glas et al., 2012, Chapter 4). This is inline with observations that the first layer of calcite precipitates at a lower rate compared to the subsequently precipitated calcite (chapter 4). Likely, Ca^{2+} -transport is being optimized with on going chamber formation until a steady-state, high rate is obtained. Therefore, we here additionally assess the effect of variable Ca^{2+} -transport and impact on El/Ca, to better explain observed banding patterns.

Because the Ca^{2+} -flux is essentially unknown, we here assume that the SOC's Ca^{2+} -concentration increases from the initial seawater concentration to 3 times the initial concentration, in gradual steps over the precipitation interval. Increasing Ca^{2+} -transport explains element banding for the different elements in our model relatively well, with more pronounced banding in Na and S (Fig. 5B) compared to constant Ca^{2+} -transport (Fig. 5A). An increase in the SOC $[\text{Ca}^{2+}]$ over time also increases the difference in Na/Ca and S/Ca between species, as a consequence of different SOC volumes, as here assumed for *A. lessonii* and *A. tepida*. The accordingly modelled correlations (linear regression: slope=0.08, intercept=3.24, $R^2=0.97$) between Na/Ca and Mg/Ca throughout the shell wall are furthermore in line with NanoSIMS observations for *A. lessonii* (Geerken et al., 2019). This relationship is similar for inter-specimen correlations as well (Geerken et al., 2018) suggesting that the variable Ca^{2+} -transport may explain co-located element banding and correlated inter-specimen variability in El/Ca.

These model results (Fig. 5B) suggest that the Ca^{2+} -transport may well not be constant over time, but is still being optimized after the onset of calcite precipitation, enhancing the amplitude in banding for all elements. This hence ‘dilutes’ the El/Ca signal by a gradually raising the Ca^{2+} -influx. An increase in El/Ca towards the end of chamber formation, which is also observed in some specimens (Geerken et al., 2019; Jonkers et al., 2016), may be explained by the final decrease in the Ca^{2+} -transport, concomitant with increasing the pH flux at the end of chamber formation, resulting in Rayleigh fractionation and hence a decrease in El/Ca for elements with a $D_{\text{El}} < 1$ (Elderfield et al., 1996)

6.5 Model pitfalls and future improvements

Modeled El/Ca values depend on the element concentrations in seawater, wall thickness, SOC volume, and inorganic partition coefficients. The first 2 variables may be easily constrained, yet the 2 latter are more difficult to obtain or essentially unknown. Furthermore, these parameters are likely to be variable, not only between species but also between specimens and even over the course of chamber formation (see also above). Validating our model’s outcome quantitatively is therefore hampered by until now ill-constrained parameters, such as the SOC volume, the inorganic D_{El} at high precipitation rates and the Ca^{2+} -influx. Moreover, additional unknown factors may play a role during biomineralization, such as organic templates, suggested to enhance nucleation (Branson et al., 2016) and phase-transformations (Jacob et al., 2017). During calcification different factors likely also affect each other dynamically. For example, the Mg concentration in the SOC likely affects Sr incorporation through lattice-distortion (Evans et al., 2015; Mewes et al., 2015) and inorganic D_{El} likely depends on the SOC El/Ca, which changes during the calcification event. Although theoretically, these mechanisms could all be taken into account, it remains challenging to still meaningfully model El/Ca when there are many unknowns. However, the model still provides constraints on hypothesized processes that may or may not explain observations, e.g. on element banding. Therefore, the effect of alternative assumptions on the model’s outcomes and potential future extensions of our model, are discussed below.

6.5.1 Trans-membrane element transport

In our model, Ca^{2+} is present at the onset of chamber formation and then transferred from the surrounding seawater into the SOC by transmembrane Ca-transport (TMT; Nehrke et al., 2013; Toyofuku et al., 2017). We assume that this influx maintains a constant $[\text{Ca}^{2+}]$ in the SOC, i.e. the influx matches the Ca^{2+} -removal by precipitation, or that $[\text{Ca}^{2+}]$ may be increased during chamber formation. It is likely, however, that other (divalent) cations are also (inadvertently) transported by the Ca^{2+} -transporter. For Sr^{2+} ,

this transport rate may be close to that of Ca^{2+} , i.e. it may be transported proportional to its ratio with Ca^{2+} in seawater. The observation that foraminiferal D_{Sr} is much higher for many foraminiferal species than foraminiferal D_{Mg} and D_{Na} (30 to 1000 times, e.g. Allen et al., 2016; Dissard et al., 2010b; Duenas-Bohorquez et al., 2011b; Elderfield et al., 2002; Raitzsch et al., 2010; Raja et al., 2005; Wit et al., 2013) supports this hypothesis. Furthermore, in chapter 4 we show that foraminiferal D_{Sr} is similar to inorganic D_{Sr} for the relatively high precipitation rates measured for *A. tepida*, which was already predicted based on inorganic precipitation experiments (Böhm et al., 2012; Tang et al., 2008) Similar to Ba, which is used in a number of labeling studies (e.g. Spero et al., 2015), Sr added to the culture medium is directly taken up into the calcite (chapter 4), showing that Sr^{2+} readily enters the SOC during calcification. The inadvertent influx of Sr^{2+} also explains the absent or at best very limited banding of Sr in foraminiferal shell walls. Whether $\text{Ca}^{2+}/\text{H}^{+}$ anti-ports occasionally transport other ions, is however, poorly constrained, but is necessary to validate the assumed lack of co-transport in this model for Na^{+} , Mg^{2+} and SO_4^{2-} . Therefore, labeling the culture medium with various elements during chamber formation (e.g. Na^{+} , Mg^{2+} , SO_4^{2-} and subsequent visualization with NanoSIMS (chapter 4) may reveal if elements may be ‘accidentally’ transported over the membrane, during chamber formation or not.

Na/Ca values predicted by the model show less variability than observed between and within specimens (Geerken et al., 2018; 2019), unless Ca^{2+} -transport is variable. Alternatively, active removal of elements from the SOC may also increase the banding amplitude. This is potentially the case for calcite-bound Na^{+} (Yoshimura et al., 2017) which is highly abundant in seawater and hence at the SOC at the onset of chamber formation. Many $\text{H}^{+}/\text{Ca}^{2+}$ -exchangers are known to transport Na-ions in addition to protons (Mulikidjanian et al., 2008; Mulikidjanian et al., 2007) due to their similar charge and the similar ionic radius between H_3O^{+} and Na^{+} (Chourasia et al., 2005). Since the ratio between $[\text{Na}^{+}]$ and $[\text{H}^{+}]$ at the onset of chamber formation is approximately 1 mol Na^{+} : $20 \cdot 10^{-9}$ mol H^{+} (at a salinity of 35 and pH of 8.1), the $[\text{Na}^{+}]$ at the SOC may be reduced as proton pumping commences and which hence would result in an overall lower mean Na/Ca and more pronounced Na banding consistent with observations. The nano-scale spatial distribution of Na may furthermore be affected by adsorption to the organic matrix (Branson et al., 2016; Branson et al., 2013; Finch and Allison, 2007; Ishikawa and Ichikuni, 1984; Yoshimura et al., 2017).

6.5.2 Partition coefficients and effect of precipitation rate

Both average El/Ca and banding therein are determined by the partitioning of elements during calcite precipitation. Here, specific values were assumed for each element, but

these values may vary given the number of parameters that influence the partition coefficient of an element. For example, the presence of organic compounds may alter the D_{El} due to its affinity for this element, which may be particularly important for El/Ca at the beginning of chamber formation. For example, Busenberg and Plummer (1985) showed that biogenic calcites, such as invertebrate shells, have a higher Na content than inorganically precipitated calcites, such as limestones or synthetic calcites (Ishikawa and Ichikuni, 1984; Kitano et al., 1975). It has been suggested that Na^+ in biogenic calcites is associated with organic components, such as polysaccharides and acidic amino acids, that are thought to lower the energy barrier that otherwise prevents calcite nucleation (Addadi and Weiner, 1985; Branson et al., 2016; White, 1978).

The model allows testing whether the effect of temperature on the inorganic D_{Mg} alone is sufficient to explain the temperature effect observed for foraminiferal Mg/Ca. With the here used parameters (Fig. 6), it appears that there must be an additional biological control on e.g. the SOC volume or the Ca^{2+} -transport is to explain the sensitivity of Mg/Ca to temperature, as previously suggested (Branson et al., 2003). However, D_{Mg} values may possibly be different at high precipitation rates, which still requires quantification.

The saturation state in the SOC is likely one of the main drivers of the CaCO_3 precipitation rate. Rates are known to affect element partitioning (e.g. Burton and Walter, 1991; Tesoriero and Pankow, 1996; Mavromatis et al., 2015), generally leading to higher coefficients at higher rates. Such relationships may be linear, exponential or sigmoidal and their effect is therefore challenging to account for in general terms. Rates of precipitation are scarce for foraminifera (chapter 4), but suggest a relatively fast precipitation compared to many inorganic precipitation experiments (e.g. Mucci and Morse, 1983). Presence of calcification inhibitors such as Mg^{2+} and SO_4^{2-} have, in turn, an effect on the precipitation rate too (e.g. Mucci and Morse, 1983), which may explain the increase of foraminiferal calcite precipitation rates over time (chapter 4). This, in turn, may suggest that partition coefficients are not static over time, but may vary with wall thickness. This is not accounted for in the model, but may be included by assuming D as a function of SOC concentrations and wall thickness.

Phase transformations may furthermore be important for element partitioning. Whether calcite forms by monomer-monomer addition (e.g. at low saturation states) or by transformation from an amorphous precursor or vaterite (Jacob et al., 2017), results in a different D_{El} (Bots et al., 2012). All of the processes described here may be used to extend the model and result in changes of the average El/Ca, patterns in banding and differences between species. Moreover, when accounting for variability in seawater chemistry (i.e.

when changing the salinity or the [El] of the seawater), modeled foraminiferal element incorporation may be used to test known dependencies and hence, proxy-relationships.

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References

- Addadi, L., Joester, D., Nudelman, F., and Weiner, S., 2006, Mollusk shell formation: a source of new concepts for understanding biomineralization processes: *Chemistry-A European Journal*, v. 12, no. 4, p. 980-987.
- Addadi, L., and Weiner, S., 1985, Interactions between acidic proteins and crystals: stereochemical requirements in biomineralization: *Proceedings of the National Academy of Sciences*, v. 82, no. 12, p. 4110-4114.
- Allen, K. A., Hönisch, B., Eggins, S. M., Haynes, L. L., Rosenthal, Y., and Yu, J., 2016, Trace element proxies for surface ocean conditions: A synthesis of culture calibrations with planktic foraminifera: *Geochimica et Cosmochimica Acta*, v. 193, p. 197-221.
- Allen, K. A., Hönisch, B., Eggins, S. M., Yu, J., Spero, H. J., and Elderfield, H., 2011, Controls on boron incorporation in cultured tests of the planktic foraminifer *Orbulina universa*: *Earth and Planetary Science Letters*, v. 309, no. 3, p. 291-301.
- Anand, P., Elderfield, H., and Conte, M. H., 2003, Calibration of Mg/Ca thermometry in planktonic foraminifera from a sediment trap time series: *Paleoceanography*, v. 18, no. 2.
- Anderson, O. R., and Faber, W. W., 1984, An estimation of calcium carbonate deposition rate in a planktonic foraminifer *Globigerinoides sacculifer* using ^{45}Ca as a tracer; a recommended procedure for improved accuracy: *The Journal of Foraminiferal Research*, v. 14, no. 4, p. 303-308.
- Angell, R. W., 1979, Calcification during chamber development in *Rosalina floridana*: *The Journal of Foraminiferal Research*, v. 9, no. 4, p. 341-353.
- Aurahs, R., Grimm, G. W., Hemleben, V., Hemleben, C., and Kucera, M., 2009, Geographical distribution of cryptic genetic types in the planktonic foraminifer *Globigerinoides ruber*: *Molecular Ecology*, v. 18, no. 8, p. 1692-1706.
- Austin, H. A., Austin, W. E., and Paterson, D. M., 2005, Extracellular cracking and content removal of the benthic diatom *Pleurosigma angulatum* (Quekett) by the benthic foraminifera *Haynesina germanica* (Ehrenberg): *Marine Micropaleontology*, v. 57, no. 3-4, p. 68-73.
- Banner, F., Sheehan, R., and Williams, E., 1973, The organic skeletons of rotaline foraminifera; a review: *The Journal of Foraminiferal Research*, v. 3, no. 1, p. 30-42.
- Barker, S., Greaves, M., and Elderfield, H., 2003, A study of cleaning procedures used for foraminiferal Mg/Ca paleothermometry: *Geochemistry, Geophysics, Geosystems*, v. 4, no. 9.
- Barras, C., Mouret, A., Nardelli, M. P., Metzger, E., Petersen, J., La, C., Filipsson, H. L., and Jorissen, F., 2018, Experimental calibration of manganese incorporation in foraminiferal calcite: *Geochimica et Cosmochimica Acta*, v. 237, p. 49-64.
- Bé, A. W., Hemleben, C., Anderson, O. R., and Spindler, M., 1979, Chamber formation in planktonic foraminifera: *Micropaleontology*, p. 294-307.
- Bemis, B. E., Spero, H. J., Bijma, J., and Lea, D. W., 1998, Reevaluation of the oxygen isotopic composition of planktonic foraminifera: Experimental results and revised paleotemperature equations: *Paleoceanography*, v. 13, no. 2, p. 150-160.
- Bentov, S., Brownlee, C., and Erez, J., 2009, The role of seawater endocytosis in the biomineralization process in calcareous foraminifera: *Proceedings of the National Academy of Sciences*, v. 106, no. 51, p. 21500-21504.
- Bentov, S., and Erez, J., 2006, Impact of biomineralization processes on the Mg content of foraminiferal shells: A biological perspective: *Geochemistry Geophysics Geosystems*, v. 7.

- Billups, K., and Schrag, D., 2002, Paleotemperatures and ice volume of the past 27 Myr revisited with paired Mg/Ca and $^{18}\text{O}/^{16}\text{O}$ measurements on benthic foraminifera: *Paleoceanography*, v. 17, no. 1, p. 3-13-11.
- Bishop, J. K., 1988, The barite-opal-organic carbon association in oceanic particulate matter: *Nature*, v. 332, no. 6162, p. 341.
- Bloemsma, M., 2015, Development of a Modelling Framework for Core Data Integration using XRF Scanning.
- Blue, C., Giuffrè, A., Mergelsberg, S., Han, N., De Yoreo, J., and Dove, P., 2017, Chemical and physical controls on the transformation of amorphous calcium carbonate into crystalline CaCO_3 polymorphs: *Geochimica et Cosmochimica Acta*, v. 196, p. 179-196.
- Böhm, F., Eisenhauer, A., Tang, J., Dietzel, M., Krabbenhöft, A., Kisakürek, B., and Horn, C., 2012, Strontium isotope fractionation of planktic foraminifera and inorganic calcite: *Geochimica et Cosmochimica Acta*, v. 93, p. 300-314.
- Bond, G., Broecker, W., Johnsen, S., McManus, J., Labeyrie, L., Jouzel, J., and Bonani, G., 1993, Correlations between climate records from North Atlantic sediments and Greenland ice: *Nature*, v. 365, no. 6442, p. 143.
- Bots, P., Benning, L. G., Rodriguez-Blanco, J.-D., Roncal-Herrero, T., and Shaw, S., 2012, Mechanistic insights into the crystallization of amorphous calcium carbonate (ACC): *Crystal Growth & Design*, v. 12, no. 7, p. 3806-3814.
- Boussetta, S., Bassinot, F., Sabbatini, A., Caillon, N., Nouet, J., Kallel, N., Rebaubier, H., Klinkhammer, G., and Labeyrie, L., 2011, Diagenetic Mg-rich calcite in Mediterranean sediments: Quantification and impact on foraminiferal Mg/Ca thermometry: *Marine Geology*, v. 280, no. 1-4, p. 195-204.
- Bowser, S. S., and Travis, J. L., 2002, Reticulopodia: structural and behavioral basis for the suprageneric placement of granuloreticulosan protists: *The Journal of Foraminiferal Research*, v. 32, no. 4, p. 440-447.
- Boyle, E., and Keigwin, L., 1985, Comparison of Atlantic and Pacific paleochemical records for the last 215,000 years: Changes in deep ocean circulation and chemical inventories: *Earth and Planetary Science Letters*, v. 76, no. 1-2, p. 135-150.
- Boyle, E. A., 1981, Cadmium, zinc, copper, and barium in foraminifera tests: *Earth and Planetary Science Letters*, v. 53, no. 1, p. 11-35.
- Branson, O., Bonnin, E. A., Perea, D. E., Spero, H. J., Zhu, Z., Winters, M., Hönisch, B., Russell, A. D., Fehrenbacher, J. S., and Gagnon, A. C., 2016, Nanometer-Scale Chemistry of a Calcite Biomineralization Template: Implications for Skeletal Composition and Nucleation: *Proceedings of the National Academy of Sciences*, p. 201522864.
- Branson, O., Kaczmarek, K., Redfern, S. A., Misra, S., Langer, G., Tylliszczak, T., Bijma, J., and Elderfield, H., 2015, The coordination and distribution of B in foraminiferal calcite: *Earth and Planetary Science Letters*, v. 416, p. 67-72.
- Branson, O., Redfern, S. A., Tylliszczak, T., Sadekov, A., Langer, G., Kimoto, K., and Elderfield, H., 2013, The coordination of Mg in foraminiferal calcite: *Earth and Planetary Science Letters*, v. 383, p. 134-141.
- Broecker, W. S., Denton, G. H., Edwards, R. L., Cheng, H., Alley, R. B., and Putnam, A. E., 2010, Putting the Younger Dryas cold event into context: *Quaternary Science Reviews*, v. 29, no. 9-10, p. 1078-1081.
- Brownlee, C., Wheeler, G. L., and Taylor, A. R., Coccolithophore biomineralization: New questions, new answers, *in* *Proceedings Seminars in cell & developmental biology* 2015, Volume 46, Elsevier, p. 11-16.

- Burton, E. A., and Walter, L. M., 1991, The effects of PCO₂ and temperature on magnesium incorporation in calcite in seawater and MgCl₂-CaCl₂ solutions: *Geochimica et Cosmochimica Acta*, v. 55, no. 3, p. 777-785.
- Busenberg, E., and Plummer, L. N., 1985, Kinetic and thermodynamic factors controlling the distribution of SO₃²⁻ and Na⁺ in calcites and selected aragonites: *Geochimica et Cosmochimica Acta*, v. 49, no. 3, p. 713-725.
- Cacho, I., Grimalt, J. O., Pelejero, C., Canals, M., Sierro, F. J., Flores, J. A., and Shackleton, N., 1999, Dansgaard-Oeschger and Heinrich event imprints in Alboran Sea paleotemperatures: *Paleoceanography*, v. 14, no. 6, p. 698-705.
- Cai, W.-J., Ma, Y., Hopkinson, B. M., Grottoli, A. G., Warner, M. E., Ding, Q., Hu, X., Yuan, X., Schoepf, V., and Xu, H., 2016, Microelectrode characterization of coral daytime interior pH and carbonate chemistry: *Nature communications*, v. 7, p. 11144.
- Calvert, S., and Fontugne, M., 2001, On the late Pleistocene-Holocene sapropel record of climatic and oceanographic variability in the eastern Mediterranean: *Paleoceanography*, v. 16, no. 1, p. 78-94.
- Carpenter, S. J., and Lohmann, K. C., 1992, Sr/Mg ratios of modern marine calcite: Empirical indicators of ocean chemistry and precipitation rate: *Geochimica et Cosmochimica Acta*, v. 56, no. 5, p. 1837-1849.
- Castañeda, I. S., Schefuß, E., Pätzold, J., Damsté, J. S. S., Weldeab, S., and Schouten, S., 2010, Millennial-scale sea surface temperature changes in the eastern Mediterranean (Nile River Delta region) over the last 27,000 years: *Paleoceanography*, v. 25, no. 1.
- Cavalier-Smith, T., 2002, The phagotrophic origin of eukaryotes and phylogenetic classification of Protozoa: *International journal of systematic and evolutionary microbiology*, v. 52, no. 2, p. 297-354.
- Chourasia, M., Sastry, G. M., and Sastry, G. N., 2005, Proton binding sites and conformational analysis of H⁺ K⁺-ATPase: *Biochemical and biophysical research communications*, v. 336, no. 3, p. 961-966.
- Culver, S. J., 1991, Early Cambrian foraminifera from west Africa: *Science*, v. 254, no. 5032, p. 689-691.
- Cushman, J. A., 1918, The foraminifera of the Atlantic Ocean, US Government Printing Office, v. 104.
- Cushman, J. A., and Wickenden, R. T. D., 1929, Recent foraminifera from off Juan Fernandez Islands: *Proceedings of the United States National Museum*.
- d'Orbigny, A. D., 1826, Tableau méthodique de la classe des Céphalopodes: *Sci. Nat.*, v. 7, p. 245-314.
- Davis, K. J., Dove, P. M., and De Yoreo, J. J., 2000, The role of Mg²⁺ as an impurity in calcite growth: *Science*, v. 290, no. 5494, p. 1134-1137.
- Davis, K. J., Dove, P. M., Wasylenki, L. E., and De Yoreo, J. J., 2004, Morphological consequences of differential Mg²⁺ incorporation at structurally distinct steps on calcite: *American Mineralogist*, v. 89, no. 5-6, p. 714-720.
- Dawber, C., and Tripathi, A., 2012, Relationships between bottom water carbonate saturation and element/Ca ratios in coretop samples of the benthic foraminifera *Oridorsalis umbonatus*: *Biogeosciences*, v. 9, no. 8, p. 3029.
- De Lange, G. J., Thomson, J., Reitz, A., Slomp, C. P., Principato, M. S., Erba, E., and Corselli, C., 2008, Synchronous basin-wide formation and redox-controlled preservation of a Mediterranean sapropel: *Nature Geoscience*, v. 1, no. 9, p. 606.
- De Nooijer, L. J., Brombacher, A., Mewes, A., Langer, G., Nehrke, G., Bijma, J., and Reichart, G.-J., 2017, Ba incorporation in benthic foraminifera: *Biogeosciences Discuss.*, p. 1-35.

- De Nooijer, L. J., Hathorne, E. C., Reichart, G. J., Langer, G., and Bijma, J., 2014a, Variability in calcitic Mg/Ca and Sr/Ca ratios in clones of the benthic foraminifer *Ammonia tepida*: *Marine Micropaleontology*, v. 107, p. 32-43.
- De Nooijer, L. J., Langer, G., Nehrke, G., and Bijma, J., 2009a, Physiological controls on seawater uptake and calcification in the benthic foraminifer *Ammonia tepida*: *Biogeosciences*, v. 6, p. 2669-2675.
- De Nooijer, L. J., Spero, H. J., Erez, J., Bijma, J., and Reichart, G. J., 2014b, Biomineralization in perforate foraminifera: *Earth-Science Reviews*, v. 135, p. 48-58.
- De Nooijer, L. J., Toyofuku, T., and Kitazato, H., 2009b, Foraminifera promote calcification by elevating their intracellular pH: *Proceedings of the National Academy of Sciences of the United States of America*, v. 106, no. 36, p. 15374-15378.
- De Yoreo, J. J., Gilbert, P. U., Sommerdijk, N. A., Penn, R. L., Whitelam, S., Joester, D., Zhang, H., Rimer, J. D., Navrotsky, A., and Banfield, J. F., 2015, Crystallization by particle attachment in synthetic, biogenic, and geologic environments: *Science*, v. 349, no. 6247, p. aaa6760.
- Dekov, V., Komy, Z., Araujo, F., Van Put, A., and Van Grieken, R., 1997, Chemical composition of sediments, suspended matter, river water and ground water of the Nile (Aswan-Sohag traverse): *Science of the total environment*, v. 201, no. 3, p. 195-210.
- Dickson, A., and Millero, F., 1987, A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media: *Deep Sea Research Part A. Oceanographic Research Papers*, v. 34, no. 10, p. 1733-1743.
- Dissard, D., Nehrke, G., Reichart, G.-J., and Bijma, J., 2010a, Impact of seawater pCO₂ on calcification and Mg/Ca and Sr/Ca ratios in benthic foraminifera calcite: results from culturing experiments with *Ammonia tepida*: *Biogeosciences*, v. 7, p. 81-93.
- Dissard, D., Nehrke, G., Reichart, G. J., and Bijma, J., 2010b, The impact of salinity on the Mg/Ca and Sr/Ca ratio in the benthic foraminifera *Ammonia tepida*: Results from culture experiments: *Geochimica Et Cosmochimica Acta*, v. 74, no. 3, p. 928-940.
- Diz, P., Barras, C., Geslin, E., Reichart, G.-J., Metzger, E., Jorissen, F., and Bijma, J., 2012, Incorporation of Mg and Sr and oxygen and carbon stable isotope fractionation in cultured *Ammonia tepida*: *Marine Micropaleontology*, v. 92, p. 16-28.
- Ducassou, E., Migeon, S., Mulder, T., Murat, A., Capotondi, L., Bernasconi, S. M., and Mascle, J., 2009, Evolution of the Nile deep-sea turbidite system during the Late Quaternary: influence of climate change on fan sedimentation: *Sedimentology*, v. 56, no. 7, p. 2061-2090.
- Dueñas-Bohórquez, A., da Rocha, R. E., Kuroyanagi, A., Bijma, J., and Reichart, G.-J., 2009, Effect of salinity and seawater calcite saturation state on Mg and Sr incorporation in cultured planktonic foraminifera: *Marine Micropaleontology*, v. 73, no. 3, p. 178-189.
- Duenas-Bohorquez, A., da Rocha, R. E., Kuroyanagi, A., de Nooijer, L. J., Bijma, J., and Reichart, G. J., 2011a, Interindividual variability and ontogenetic effects on Mg and Sr incorporation in the planktonic foraminifer *Globigerinoides sacculifer*: *Geochimica Et Cosmochimica Acta*, v. 75, no. 2, p. 520-532.
- Duenas-Bohorquez, A., Raitzsch, M., de Nooijer, L. J., and Reichart, G. J., 2011b, Independent impacts of calcium and carbonate ion concentration on Mg and Sr incorporation in cultured benthic foraminifera: *Marine Micropaleontology*, v. 81, no. 3-4, p. 122-130.
- Duguay, L. E., and Taylor, D. L., 1978, Primary production and calcification by the soritid foraminifer *Archais angulatus* (Fichtel & Moll): *The Journal of Protozoology*, v. 25, no. 3, p. 356-361.
- Dymond, J., and Collier, R., 1996, Particulate barium fluxes and their relationships to biological productivity: *Deep Sea Research Part II: Topical Studies in Oceanography*, v. 43, no. 4-6, p. 1283-1308.

- Eggins, S., De Deckker, P., and Marshall, J., 2003, Mg/Ca variation in planktonic foraminifera tests: implications for reconstructing palaeo-seawater temperature and habitat migration: *Earth and Planetary Science Letters*, v. 212, no. 3-4, p. 291-306.
- Eggins, S. M., Sadekov, A., and De Deckker, P., 2004, Modulation and daily banding of Mg/Ca in *Orbulina universa* tests by symbiont photosynthesis and respiration: a complication for seawater thermometry?: *Earth and Planetary Science Letters*, v. 225, no. 3-4, p. 411-419.
- Elderfield, H., Bertram, C. J., and Erez, J., 1996, Biomineralization model for the incorporation of trace elements into foraminiferal calcium carbonate: *Earth and Planetary Science Letters*, v. 142, no. 3-4, p. 409-423.
- Elderfield, H., and Ganssen, G., 2000, Past temperature and delta O-18 of surface ocean waters inferred from foraminiferal Mg/Ca ratios: *Nature*, v. 405, no. 6785, p. 442-445.
- Elderfield, H., Vautravers, M., and Cooper, M., 2002, The relationship between shell size and Mg/Ca, Sr/Ca, delta O-18, and delta C-13 of species of planktonic foraminifera: *Geochemistry Geophysics Geosystems*, v. 3.
- Emeis, K.-C., Struck, U., Schulz, H.-M., Rosenberg, R., Bernasconi, S., Erlenkeuser, H., Sakamoto, T., and Martinez-Ruiz, F., 2000, Temperature and salinity variations of Mediterranean Sea surface waters over the last 16,000 years from records of planktonic stable oxygen isotopes and alkenone unsaturation ratios: *Palaeogeography, Palaeoclimatology, Palaeoecology*, v. 158, no. 3-4, p. 259-280.
- Emeis, K. C., Schulz, H., Struck, U., Rossignol-Strick, M., Erlenkeuser, H., Howell, M., Kroon, D., Mackensen, A., Ishizuka, S., and Oba, T., 2003, Eastern Mediterranean surface water temperatures and $\delta^{18}\text{O}$ composition during deposition of sapropels in the late Quaternary: *Paleoceanography*, v. 18, no. 1.
- Emiliani, C., 1954, Depth habitats of some species of pelagic foraminifera as indicated by oxygen isotope ratios: *American Journal of Science*, v. 252, no. 3, p. 149-158.
- Emiliani, C., 1955, Pleistocene temperatures: *The Journal of Geology*, v. 63, no. 6, p. 538-578.
- Erez, J., 1983, Calcification rates, photosynthesis and light in planktonic foraminifera, Biomineralization and biological metal accumulation, Springer, p. 307-312.
- Erez, J., 2003, The source of ions for biomineralization in foraminifera and their implications for paleoceanographic proxies: *Biomineralization*, v. 54, p. 115-149.
- Ernst, S., Janse, M., Renema, W., Kouwenhoven, T., Goudeau, M. L., and Reichart, G. J., 2011, Benthic foraminifera in a large indo-pacific coral reef aquarium: *Journal of Foraminiferal Research*, v. 41, no. 2, p. 101-113.
- Evans, D., Erez, J., Oron, S., and Müller, W., 2015, Mg/Ca-temperature and seawater-test chemistry relationships in the shallow-dwelling large benthic foraminifera *Operculina ammonoides*: *Geochimica et Cosmochimica Acta*, v. 148, p. 325-342.
- Evans, D., Müller, W., and Erez, J., 2018, Assessing foraminifera biomineralisation models through trace element data of cultures under variable seawater chemistry: *Geochimica et Cosmochimica Acta*.
- Fehrenbacher, J. S., Russell, A. D., Davis, C. V., Gagnon, A. C., Spero, H. J., Cliff, J. B., Zhu, Z., and Martin, P., 2017, Link between light-triggered Mg-banding and chamber formation in the planktic foraminifera *Neoglobobulimina dutertrei*: Pacific Northwest National Laboratory (PNNL), Richland, WA (US), Environmental Molecular Sciences Laboratory (EMSL).
- Ferguson, J., Henderson, G., Kucera, M., and Rickaby, R., 2008, Systematic change of foraminiferal Mg/Ca ratios across a strong salinity gradient: *Earth and Planetary Science Letters*, v. 265, no. 1-2, p. 153-166.

- Finch, A., and Allison, N., 2007, Coordination of Sr and Mg in calcite and aragonite: *Mineralogical Magazine*, v. 71, no. 5, p. 539-552.
- Fontugne, M. R., and Calvert, S. E., 1992, Late Pleistocene variability of the carbon isotopic composition of organic matter in the eastern Mediterranean: monitor of changes in carbon sources and atmospheric CO₂ concentrations: *Paleoceanography*, v. 7, no. 1, p. 1-20.
- Gabitov, R., Gagnon, A., Guan, Y., Eiler, J., and Adkins, J., 2013, Accurate Mg/Ca, Sr/Ca, and Ba/Ca ratio measurements in carbonates by SIMS and NanoSIMS and an assessment of heterogeneity in common calcium carbonate standards: *Chemical Geology*, v. 356, p. 94-108.
- Gagnon, A. C., Adkins, J. F., Fernandez, D. P., and Robinson, L. F., 2007, Sr/Ca and Mg/Ca vital effects correlated with skeletal architecture in a scleractinian deep-sea coral and the role of Rayleigh fractionation: *Earth and Planetary Science Letters*, v. 261, no. 1-2, p. 280-295.
- Ganssen, G., and Troelstra, S. R., 1987, Paleoenvironmental changes from stable isotopes in planktonic foraminifera from eastern Mediterranean sapropels: *Marine Geology*, v. 75, no. 1-4, p. 221-230.
- Gat, J. R., Shemesh, A., Tziperman, E., Hecht, A., Georgopoulos, D., and Basturk, O., 1996, The stable isotope composition of waters of the eastern Mediterranean Sea: *Journal of Geophysical Research: Oceans*, v. 101, no. C3, p. 6441-6451.
- Geerken, E., de Nooijer, L., Roepert, A., Polerecky, L., King, H., and Reichart, G., 2019, Element banding and organic linings within chamber walls of two benthic foraminifera: *Scientific reports*, v. 9, no. 1, p. 3598.
- Geerken, E., de Nooijer, L. J., van Dijk, I., and Reichart, G.-J., 2018, Impact of salinity on element incorporation in two benthic foraminiferal species with contrasting magnesium contents: *Biogeosciences*, v. 15, no. 7, p. 2205.
- Giuffrè, A. J., Gagnon, A. C., De Yoreo, J. J., and Dove, P. M., 2015, Isotopic tracer evidence for the amorphous calcium carbonate to calcite transformation by dissolution-reprecipitation: *Geochimica et Cosmochimica Acta*, v. 165, p. 407-417.
- Glas, M. S., Langer, G., and Keul, N., 2012, Calcification acidifies the microenvironment of a benthic foraminifer (*Ammonia* sp.): *Journal of Experimental Marine Biology and Ecology*, v. 424, p. 53-58.
- Glock, N., Schönfeld, J., and Mallon, J., 2012, The functionality of pores in benthic foraminifera in view of bottom water oxygenation: a review, *Anoxia*, Springer, p. 537-552.
- Grant, K., Grimm, R., Mikolajewicz, U., Marino, G., Ziegler, M., and Rohling, E., 2016, The timing of Mediterranean sapropel deposition relative to insolation, sea-level and African monsoon changes: *Quaternary Science Reviews*, v. 140, p. 125-141.
- Grant, K., Rohling, E., Bar-Matthews, M., Ayalon, A., Medina-Elizalde, M., Ramsey, C. B., Satow, C., and Roberts, A., 2012, Rapid coupling between ice volume and polar temperature over the past 150,000 years: *Nature*, v. 491, no. 7426, p. 744.
- Grant, K., Rohling, E., Ramsey, C. B., Cheng, H., Edwards, R., Florindo, F., Heslop, D., Marra, F., Roberts, A., and Tamsiea, M. E., 2014, Sea-level variability over five glacial cycles: *Nature communications*, v. 5, p. 5076.
- Gray, W. R., Weldeab, S., Lea, D. W., Rosenthal, Y., Gruber, N., Donner, B., and Fischer, G., 2018, The effects of temperature, salinity, and the carbonate system on Mg/Ca in *Globigerinoides ruber* (white): A global sediment trap calibration: *Earth and Planetary Science Letters*, v. 482, p. 607-620.
- Groeneveld, J., and Filipsson, H., 2013, Mg/Ca and Mn/Ca ratios in benthic foraminifera: the potential to reconstruct past variations in temperature and hypoxia in shelf regions: *Biogeosciences*, v. 10, no. 7, p. 5125-5138.

- Guillong, M., Meier, D. L., Allan, M. M., Heinrich, C. A., and Yardley, B. W., 2008, Appendix A6: SILLS: A MATLAB-based program for the reduction of laser ablation ICP-MS data of homogeneous materials and inclusions: Mineralogical Association of Canada Short Course, v. 40, p. 328-333.
- Gupta, B. K. S., Smith, L. E., and Lobegeier, M. K., 2007, Attachment of Foraminifera to vestimentiferan tubeworms at cold seeps: refuge from seafloor hypoxia and sulfide toxicity: *Marine Micropaleontology*, v. 62, no. 1, p. 1-6.
- Hansen, J., Sato, M., Kharecha, P., Von Schuckmann, K., Beerling, D. J., Cao, J., Marcott, S., Masson-Delmotte, V., Prather, M. J., and Rohling, E. J., 2016, Young people's burden: requirement of negative CO₂ emissions: arXiv preprint arXiv:1609.05878.
- Hasenfratz, A. P., Schiebel, R., Thornalley, D. J., Schönfeld, J., Jaccard, S. L., Martínez-García, A., Holbourn, A., Jennings, A. E., Kuhnt, W., and Lear, C. H., 2017, Mg/Ca-temperature calibration for the benthic foraminifera *Melonis barleeanum* and *Melonis pompilioides*: *Geochimica et cosmochimica acta*, v. 217, p. 365-383.
- Hathorne, E. C., James, R. H., and Lampitt, R. S., 2009, Environmental versus biomineralization controls on the intratest variation in the trace element composition of the planktonic foraminifera *G. inflata* and *G. scitula*: *Paleoceanography*, v. 24.
- Hathorne, E. C., James, R. H., Savage, P., and Alard, O., 2008, Physical and chemical characteristics of particles produced by laser ablation of biogenic calcium carbonate: *Journal of Analytical Atomic Spectrometry*, v. 23, no. 2, p. 240-243.
- Hauzer, H., Evans, D., Müller, W., Rosenthal, Y., and Erez, J., 2018, Calibration of Na partitioning in the calcitic foraminifer *Operculina ammonoides* under variable Ca concentration: Toward reconstructing past seawater composition: *Earth and Planetary Science Letters*, v. 497, p. 80-91.
- Hays, J. D., Imbrie, J., and Shackleton, N. J., 1976, Variations in the Earth's orbit: pacemaker of the ice ages: *Science*, v. 194, no. 4270, p. 1121-1132.
- Hayward, B. W., Holzmann, M., Grenfell, H. R., Pawlowski, J., and Triggs, C. M., 2004, Morphological distinction of molecular types in *Ammonia*—towards a taxonomic revision of the world's most commonly misidentified foraminifera: *Marine Micropaleontology*, v. 50, no. 3-4, p. 237-271.
- Helz, G., Miller, C., Charnock, J., Mosselmans, J., Patrick, R., Garner, C., and Vaughan, D., 1996, Mechanism of molybdenum removal from the sea and its concentration in black shales: EXAFS evidence: *Geochimica et Cosmochimica Acta*, v. 60, no. 19, p. 3631-3642.
- Hemleben, C., Be, A. W., Anderson, O. R., and Tuntivate, S., 1977, Test morphology, organic layers and chamber formation of the planktonic foraminifer *Globorotalia menardii* (d'Orbigny): *The Journal of Foraminiferal Research*, v. 7, no. 1, p. 1-25.
- Hemleben, C., Erson, O., Berthold, W., and Spindler, M., 1986, Calcification and chamber formation in Foraminifera—a brief overview, p. 237-249.
- Hemleben, C., Spindler, M., and Anderson, O. R., 2012, *Modern planktonic foraminifera*, Springer Science & Business Media.
- Hemming, N., and Hanson, G., 1992, Boron isotopic composition and concentration in modern marine carbonates: *Geochimica et Cosmochimica Acta*, v. 56, no. 1, p. 537-543.
- Hennekam, R., Sweere, T., Tjallingii, R., de Lange, G. J., and Reichert, G.-J., 2019, Trace metal analysis of sediment cores using a novel X-ray fluorescence core scanning method: *Quaternary International*, v. 514, p. 55-67.

- Holland, K., Eggins, S. M., Hönisch, B., Haynes, L. L., and Branson, O., 2017, Calcification rate and shell chemistry response of the planktic foraminifer *Orbulina universa* to changes in microenvironment seawater carbonate chemistry: *Earth and Planetary Science Letters*, v. 464, p. 124-134.
- Hönisch, B., Allen, K. A., Lea, D. W., Spero, H. J., Eggins, S. M., Arbuszewski, J., deMenocal, P., Rosenthal, Y., Russell, A. D., and Elderfield, H., 2013, The influence of salinity on Mg/Ca in planktic foraminifers—Evidence from cultures, core-top sediments and complementary $\delta^{18}\text{O}$: *Geochimica et Cosmochimica Acta*, v. 121, p. 196-213.
- Hönisch, B., Allen, K. A., Russell, A. D., Eggins, S. M., Bijma, J., Spero, H. J., Lea, D. W., and Yu, J., 2011, Planktic foraminifers as recorders of seawater Ba/Ca: *Marine Micropaleontology*, v. 79, no. 1-2, p. 52-57.
- Hoogakker, B. A., Klinkhammer, G. P., Elderfield, H., Rohling, E. J., and Hayward, C., 2009, Mg/Ca paleothermometry in high salinity environments: *Earth and Planetary Science Letters*, v. 284, no. 3-4, p. 583-589.
- Huang, Y., Clemens, S. C., Liu, W., Wang, Y., and Prell, W. L., 2007, Large-scale hydrological change drove the late Miocene C4 plant expansion in the Himalayan foreland and Arabian Peninsula: *Geology*, v. 35, no. 6, p. 531-534.
- Imbrie, J., Hays, J. D., Martinson, D. G., McIntyre, A., Mix, A. C., Morley, J. J., Pisias, N. G., Prell, W. L., and Shackleton, N. J., 1984, The orbital theory of Pleistocene climate: support from a revised chronology of the marine $\delta^{18}\text{O}$ record.
- Ishikawa, M., and Ichikuni, M., 1984, Uptake of sodium and potassium by calcite: *Chemical geology*, v. 42, no. 1-4, p. 137-146.
- Jacob, D., Wirth, R., Agbaje, O., Branson, O., and Eggins, S., 2017, Planktic foraminifera form their shells via metastable carbonate phases: *Nature Communications*, v. 8, no. 1, p. 1265.
- Jonkers, L., Buse, B., Brummer, G.-J. A., and Hall, I. R., 2016, Chamber formation leads to Mg/Ca banding in the planktonic foraminifer *Neogloboquadrina pachyderma*: *Earth and Planetary Science Letters*, v. 451, p. 177-184.
- Jorissen, F. J., 1999, Benthic foraminiferal microhabitats below the sediment-water interface, *Modern foraminifera*, Springer, p. 161-179.
- Kasper, S., Van Der Meer, M., Mets, A., Zahn, R., Sinninghe Damsté, J., and Schouten, S., 2014, Salinity changes in the Agulhas leakage area recorded by stable hydrogen isotopes of C 37 alkenones during Termination I and II: *Climate of the Past*, v. 10, no. 1, p. 251-260.
- Keul, N., Langer, G., de Nooijer, L. J., and Bijma, J., 2013a, Effect of ocean acidification on the benthic foraminifera *Ammonia* sp. is caused by a decrease in carbonate ion concentration: *Biogeosciences*, v. 10, no. 10, p. 6185-6198.
- Keul, N., Langer, G., de Nooijer, L. J., Nehrke, G., Reichert, G. J., and Bijma, J., 2013b, Incorporation of uranium in benthic foraminiferal calcite reflects seawater carbonate ion concentration: *Geochemistry Geophysics Geosystems*, v. 14, no. 1, p. 102-111.
- Keul, N., Langer, G., Thoms, S., de Nooijer, L. J., Reichert, G.-J., and Bijma, J., 2017, Exploring foraminiferal Sr/Ca as a new carbonate system proxy: *Geochimica et Cosmochimica Acta*, v. 202, p. 374-386.
- Kim, Y.-Y., Carloni, J. D., Demarchi, B., Sparks, D., Reid, D. G., Kunitake, M. E., Tang, C. C., Duer, M. J., Freeman, C. L., and Pokroy, B., 2016, Tuning hardness in calcite by incorporation of amino acids: *Nature materials*, v. 15, no. 8, p. 903.
- Kisakürek, B., Eisenhauer, A., Böhm, F., Garbe-Schönberg, D., and Erez, J., 2008, Controls on shell Mg/Ca and Sr/Ca in cultured planktonic foraminifera, *Globigerinoides ruber* (white): *Earth and Planetary Science Letters*, v. 273, no. 3, p. 260-269.

- Kitano, Y., Okumura, M., and Idogaki, M., 1975, Incorporation of sodium, chloride and sulfate with calcium carbonate: *Geochemical Journal*, v. 9, no. 2, p. 75-84.
- Klinkhammer, G. P., Mix, A. C., and Haley, B., 2009, Increased dissolved terrestrial input to the coastal ocean during the last deglaciation: *Geochemistry, Geophysics, Geosystems*, v. 10, no. 3.
- Knoll, A. H., 2003, Biomineralization and evolutionary history: Reviews in mineralogy and geochemistry, v. 54, no. 1, p. 329-356.
- Koho, K., de Nooijer, L., and Reichart, G., 2015, Combining benthic foraminiferal ecology and shell Mn/Ca to deconvolve past bottom water oxygenation and paleoproductivity: *Geochimica et Cosmochimica Acta*, v. 165, p. 294-306.
- Koho, K. A., De Nooijer, L. J., Fontanier, C., Toyofuku, T., Oguri, K., Kitazato, H., and Reichart, G.-J., 2017, Benthic foraminiferal Mn/Ca ratios reflect microhabitat preferences: *Biogeosciences*, v. 14, no. 12, p. 3067.
- Kontakiotis, G., Mortyn, P. G., Antonarakou, A., Martínez-Botí, M. A., and Triantaphyllou, M. V., 2011, Field-based validation of a diagenetic effect on *G. ruber* Mg/Ca paleothermometry: Core top results from the Aegean Sea (eastern Mediterranean): *Geochemistry, Geophysics, Geosystems*, v. 12, no. 9.
- Kunioka, D., Shirai, K., Takahata, N., Sano, Y., Toyofuku, T., and Ujiie, Y., 2006, Microdistribution of Mg/Ca, Sr/Ca, and Ba/Ca ratios in *Pulleniatina obliquiloculata* test by using a NanoSIMS: Implication for the vital effect mechanism: *Geochemistry Geophysics Geosystems*, v. 7.
- Lea, D. W., Mashiotto, T. A., and Spero, H. J., 1999, Controls on magnesium and strontium uptake in planktonic foraminifera determined by live culturing: *Geochimica et Cosmochimica Acta*, v. 63, no. 16, p. 2369-2379.
- Lea, D. W., and Spero, H. J., 1994, Assessing the reliability of paleochemical tracers: Barium uptake in the shells of planktonic foraminifera: *Paleoceanography*, v. 9, no. 3, p. 445-452.
- Lear, C., Elderfield, H., and Wilson, P., 2000, Cenozoic deep-sea temperatures and global ice volumes from Mg/Ca in benthic foraminiferal calcite: *Science*, v. 287, no. 5451, p. 269-272.
- Lear, C. H., Rosenthal, Y., and Slowey, N., 2002, Benthic foraminiferal Mg/Ca-paleothermometry: A revised core-top calibration: *Geochimica et Cosmochimica Acta*, v. 66, no. 19, p. 3375-3387.
- Lenton, T. M., Held, H., Kriegler, E., Hall, J. W., Lucht, W., Rahmstorf, S., and Schellnhuber, H. J., 2008, Tipping elements in the Earth's climate system: *Proceedings of the national Academy of Sciences*, v. 105, no. 6, p. 1786-1793.
- Littlewood, J. L., Shaw, S., Peacock, C. L., Bots, P., Trivedi, D., and Burke, I. T., 2017, Mechanism of Enhanced Strontium Uptake into Calcite via an Amorphous Calcium Carbonate Crystallization Pathway: *Crystal Growth & Design*, v. 17, no. 3, p. 1214-1223.
- Lorens, R. B., 1981, Sr, Cd, Mn and Co distribution coefficients in calcite as a function of calcite precipitation rate: *Geochimica et Cosmochimica Acta*, v. 45, no. 4, p. 553-561.
- Lourens, L. J., Wehausen, R., and Brumsack, H. J., 2001, Geological constraints on tidal dissipation and dynamical ellipticity of the Earth over the past three million years: *Nature*, v. 409, no. 6823, p. 1029.
- Lowenstam, H. A., and Weiner, S., 1989, *On biomineralization*, Oxford University Press on Demand.
- Marchitto, T., Bryan, S., Doss, W., McCulloch, M., and Montagna, P., 2018, A simple biomineralization model to explain Li, Mg, and Sr incorporation into aragonitic foraminifera and corals: *Earth and Planetary Science Letters*, v. 481, p. 20-29.
- Martrat, B., Grimalt, J. O., Shackleton, N. J., de Abreu, L., Hutterli, M. A., and Stocker, T. F., 2007, Four climate cycles of recurring deep and surface water destabilizations on the Iberian margin: *Science*, v. 317, no. 5837, p. 502-507.

- Mathien-Blard, E., and Bassinot, F., 2009, Salinity bias on the foraminifera Mg/Ca thermometry: Correction procedure and implications for past ocean hydrographic reconstructions: *Geochemistry, Geophysics, Geosystems*, v. 10, no. 12.
- Mavromatis, V., Montouillout, V., Noireaux, J., Gaillardet, J., and Schott, J., 2015, Characterization of boron incorporation and speciation in calcite and aragonite from co-precipitation experiments under controlled pH, temperature and precipitation rate: *Geochimica et cosmochimica acta*, v. 150, p. 299-313.
- Mazza, P. P., Lovari, S., Masini, F., Masseti, M., and Rustioni, M., 2013, A multidisciplinary approach to the analysis of multifactorial land mammal colonization of islands: *BioScience*, v. 63, no. 12, p. 939-951.
- Mehrbach, C., Culberson, C., Hawley, J., and Pytkowicz, R., 1973, Measurement of the apparent dissociation constants of carbonic acid in seawater at atmospheric pressure: *Limnology and Oceanography*, v. 18, no. 6, p. 897-907.
- Mewes, A., Langer, G., de Nooijer, L. J., Bijma, J., and Reichart, G. J., 2014, Effect of different seawater Mg²⁺ concentrations on calcification in two benthic foraminifers *Marine Micropaleontology*, v. 113, p. 56-64.
- Mewes, A., Langer, G., Reichart, G.-J., de Nooijer, L. J., Nehrke, G., and Bijma, J., 2015, The impact of Mg contents on Sr partitioning in benthic foraminifers: *Chemical Geology*, v. 412, p. 92-98.
- Mezger, E., de Nooijer, L., Siccha, M., Brummer, G. J., Kucera, M., and Reichart, G. J., 2018, Taphonomic and ontogenetic effects on Na/Ca and Mg/Ca in spinose planktonic foraminifera from the Red Sea: *Geochemistry, Geophysics, Geosystems*, v. 19, no. 11, p. 4174-4194.
- Mezger, E., Nooijer, L., Boer, W., Brummer, G., and Reichart, G., 2016, Salinity controls on Na incorporation in Red Sea planktonic foraminifera: *Paleoceanography*.
- Morard, R., Vollmar, N. M., Greco, M., and Kucera, M., 2019, Unassigned diversity of planktonic foraminifera from environmental sequencing revealed as known but neglected species: *PloS one*, v. 14, no. 3, p. e0213936.
- Morse, J. W., Arvidson, R. S., and Lüttge, A., 2007, Calcium carbonate formation and dissolution: *Chemical reviews*, v. 107, no. 2, p. 342-381.
- Mucci, A., 1986, Growth kinetics and composition of magnesian calcite overgrowths precipitated from seawater: Quantitative influence of orthophosphate ions: *Geochimica et Cosmochimica Acta*, v. 50, no. 10, p. 2255-2265.
- Mucci, A., and Morse, J. W., 1983, The incorporation of Mg²⁺ and Sr²⁺ into calcite overgrowths: influences of growth rate and solution composition: *Geochimica et Cosmochimica Acta*, v. 47, no. 2, p. 217-233.
- Mulkidjanian, A. Y., Galperin, M. Y., Makarova, K. S., Wolf, Y. I., and Koonin, E. V., 2008, Evolutionary primacy of sodium bioenergetics: *Biology direct*, v. 3, no. 1, p. 13.
- Mulkidjanian, A. Y., Makarova, K. S., Galperin, M. Y., and Koonin, E. V., 2007, Inventing the dynamo machine: the evolution of the F-type and V-type ATPases: *Nature Reviews Microbiology*, v. 5, no. 11, p. 892-899.
- Munsell, D., Kramar, U., Dissard, D., Nehrke, G., Berner, Z., Bijma, J., Reichart, G.-J., and Neumann, T., 2010, Heavy metal incorporation in foraminiferal calcite: results from multi-element enrichment culture experiments with *Ammonia tepida*: *Biogeosciences*, v. 7, no. 8, p. 2339-2350.
- Murray, J. W., 2007, Biodiversity of living benthic foraminifera: How many species are there?: *Marine Micropaleontology*, v. 64, no. 3-4, p. 163-176.
- , 2014, *Ecology and palaeoecology of benthic foraminifera*, Routledge.

- Nagai, Y., Uematsu, K., Chen, C., Wani, R., Tyszka, J., and Toyofuku, T., 2018a, Weaving of biomineralization framework in rotaliid foraminifera: implications for paleoceanographic proxies: *Biogeosciences*, v. 15, no. 22, p. 6773.
- Nagai, Y., Uematsu, K., Wani, R., and Toyofuku, T., 2018b, Reading the Fine Print: Ultra-Microstructures of Foraminiferal Calcification Revealed Using Focused Ion Beam Microscopy: *Frontiers in Marine Science*, v. 5, p. 67.
- Nakajima, K., Nagai, Y., Suzuki, M., Oaki, Y., Naito, K., Tanaka, Y., Toyofuku, T., and Imai, H., 2016, Mesoscopic crystallographic textures on shells of a hyaline radial foraminifer *Ammonia beccarii*: *CrystEngComm*, v. 18, no. 37, p. 7135-7139.
- Nehrke, G., Keul, N., Langer, G., de Nooijer, L. J., Bijma, J., and Meibom, A., 2013, A new model for biomineralization and trace-element signatures of Foraminifera tests: *Biogeosciences*, v. 10, no. 10, p. 6759-6767.
- Nehrke, G., Reichart, G.-J., Van Cappellen, P., Meile, C., and Bijma, J., 2007, Dependence of calcite growth rate and Sr partitioning on solution stoichiometry: non-Kossel crystal growth: *Geochimica et Cosmochimica Acta*, v. 71, no. 9, p. 2240-2249.
- Ní Fhlaithearta, S., Ernst, S. R., Nierop, K. G., de Lange, G. J., and Reichart, G.-J., 2013, Molecular and isotopic composition of foraminiferal organic linings: *Marine Micropaleontology*, v. 102, p. 69-78.
- Ní Fhlaithearta, S., Reichart, G. J., Jorissen, F. J., Fontanier, C., Rohling, E., Thomson, J., and De Lange, G., 2010, Reconstructing the seafloor environment during sapropel formation using benthic foraminiferal trace metals, stable isotopes, and sediment composition: *Paleoceanography*, v. 25, no. 4.
- Nürnberg, D., Bijma, J., and Hemleben, C., 1996, Assessing the reliability of magnesium in foraminiferal calcite as a proxy for water mass temperatures: *Geochimica et Cosmochimica Acta*, v. 60, no. 5, p. 803-814.
- Osborne, A. H., Marino, G., Vance, D., and Rohling, E., 2010, Eastern Mediterranean surface water Nd during Eemian sapropel S5: monitoring northerly (mid-latitude) versus southerly (sub-tropical) freshwater contributions: *Quaternary Science Reviews*, v. 29, no. 19-20, p. 2473-2483.
- Paquette, J., and Reeder, R. J., 1990, Single-crystal X-ray structure refinements of two biogenic magnesian calcite crystals: *American Mineralogist*, v. 75, no. 9-10, p. 1151-1158.
- Paquette, J., and Reeder, R. J., 1995, Relationship between surface structure, growth mechanism, and trace element incorporation in calcite: *Geochimica et Cosmochimica Acta*, v. 59, no. 4, p. 735-749.
- Paris, G., Fehrenbacher, J. S., Sessions, A. L., Spero, H. J., and Adkins, J. F., 2014, Experimental determination of carbonate-associated sulfate delta S-34 in planktonic foraminifera shells: *Geochemistry Geophysics Geosystems*, v. 15, no. 4, p. 1452-1461.
- Pawlowski, J., Holzmann, M., Berney, C., Fahrni, J., Gooday, A. J., Cedhagen, T., Habura, A., and Bowser, S. S., 2003, The evolution of early Foraminifera: *Proceedings of the National Academy of Sciences*, v. 100, no. 20, p. 11494-11498.
- Pena, L., Calvo, E., Cacho, I., Eggins, S., and Pelejero, C., 2005, Identification and removal of Mn-Mg-rich contaminant phases on foraminiferal tests: Implications for Mg/Ca past temperature reconstructions: *Geochemistry, Geophysics, Geosystems*, v. 6, no. 9.
- Petersen, J., Barras, C., Bézous, A., La, C., de Nooijer, L. J., Meysman, F. J., Mouret, A., Slomp, C. P., and Jorissen, F. J., 2018, Mn/Ca intra-and inter-test variability in the benthic foraminifer *Ammonia tepida*: *Biogeosciences*, v. 15, no. 1, p. 331.
- Petit, J.-R., Jouzel, J., Raynaud, D., Barkov, N. I., Barnola, J.-M., Basile, I., Bender, M., Chappellaz, J., Davis, M., and Delaygue, G., 1999, Climate and atmospheric history of the past 420,000 years from the Vostok ice core, Antarctica: *Nature*, v. 399, no. 6735, p. 429.

- Pierre, C., 1999, The oxygen and carbon isotope distribution in the Mediterranean water masses: *Marine geology*, v. 153, no. 1-4, p. 41-55.
- Pingitore Jr, N. E., and Eastman, M. P., 1984, The experimental partitioning of Ba²⁺ into calcite: *Chemical Geology*, v. 45, no. 1-2, p. 113-120.
- Pingitore Jr, N. E., and Eastman, 1986, The coprecipitation of Sr²⁺ with calcite at 25 C and 1 atm: *Geochimica et Cosmochimica Acta*, v. 50, no. 10, p. 2195-2203.
- Polerecky, L., Adam, B., Milucka, J., Musat, N., Vagner, T., and Kuypers, M. M., 2012, Look@ NanoSIMS—a tool for the analysis of nanoSIMS data in environmental microbiology: *Environmental microbiology*, v. 14, no. 4, p. 1009-1023.
- Raitzsch, M., Duenas-Bohorquez, A., Reichart, G. J., de Nooijer, L. J., and Bickert, T., 2010, Incorporation of Mg and Sr in calcite of cultured benthic foraminifera: impact of calcium concentration and associated calcite saturation state: *Biogeosciences*, v. 7, no. 3, p. 869-881.
- Raja, R., Saraswati, P. K., and Iwao, K., 2007, A field-based study on variation in Mg/Ca and Sr/Ca in larger benthic foraminifera: *Geochemistry, Geophysics, Geosystems*, v. 8, no. 10.
- Raja, R., Saraswati, P. K., Rogers, K., and Iwao, K., 2005, Magnesium and strontium compositions of recent symbiont-bearing benthic foraminifera: *Marine Micropaleontology*, v. 58, no. 1, p. 31-44.
- Reichart, G. J., Jorissen, F., Anschutz, P., and Mason, P. R. D., 2003, Single foraminiferal test chemistry records the marine environment: *Geology*, v. 31, no. 4, p. 355-358.
- Reiss, Z., 1957, The *Bilamellidea*, nov. superfam., and remarks on Cretaceous *globorotaliids*: *Contrib Cushman Found Foraminifera*, v. 8, p. 127-145.
- Reiss, Z., 1960, Structure of so-called Eponides and some other rotaliiform foraminifera, State of Israel, Ministry of Development, Geological Survey.
- Revel, M., Ducassou, E., Grousset, F., Bernasconi, S., Migeon, S., Révillon, S., Mascle, J., Murat, A., Zaragosi, S., and Bosch, D., 2010, 100,000 years of African monsoon variability recorded in sediments of the Nile margin: *Quaternary Science Reviews*, v. 29, no. 11-12, p. 1342-1362.
- Ridgwell, A., 2005, A Mid Mesozoic Revolution in the regulation of ocean chemistry: *Marine Geology*, v. 217, no. 3-4, p. 339-357.
- Ridgwell, A., and Zeebe, R. E., 2005, The role of the global carbonate cycle in the regulation and evolution of the Earth system: *Earth and Planetary Science Letters*, v. 234, no. 3-4, p. 299-315.
- Rimstidt, J. D., Balog, A., and Webb, J., 1998, Distribution of trace elements between carbonate minerals and aqueous solutions: *Geochimica et Cosmochimica Acta*, v. 62, no. 11, p. 1851-1863.
- Rogelj, J., D. Shindell, K. Jiang, S. Fifita, P. Forster, V. Ginzburg, C. Handa, H. Khesghi, S. Kobayashi, E. Kriegler, L. Mundaca, R. Séférián, and M.V. Vilariño, in press, Mitigation Pathways Compatible with 1.5°C in the Context of Sustainable Development. In Press.: *Global Warming of 1.5°C. An IPCC Special Report on the impacts of global warming of 1.5°C above pre-industrial levels and related global greenhouse gas emission pathways, in the context of strengthening the global response to the threat of climate change, sustainable development, and efforts to eradicate poverty* [Masson-Delmotte, V., P. Zhai, H.-O. Pörtner, D. Roberts, J. Skea, P.R. Shukla, A. Pirani, W. Moufouma-Okia, C. Péan, R. Pidcock, S. Connors, J.B.R. Matthews, Y. Chen, X. Zhou, M.I. Gomis, E. Lonnoy, T. Maycock, M. Tignor, and T. Waterfield (eds.)].
- Rohling, E., Cane, T., Cooke, S., Sprovieri, M., Bouloubassi, I., Emeis, K., Schiebel, R., Kroon, D., Jorissen, F., and Lorre, A., 2002, African monsoon variability during the previous interglacial maximum: *Earth and Planetary Science Letters*, v. 202, no. 1, p. 61-75.
- Rohling, E., Foster, G. L., Grant, K., Marino, G., Roberts, A., Tamisiea, M. E., and Williams, F., 2014, Sea-level and deep-sea-temperature variability over the past 5.3 million years: *Nature*, v. 508, no. 7497, p. 477.

- Rohling, E., and Hilgen, F., 2007, The eastern Mediterranean climate at times of sapropel formation: a review: *Netherlands Journal of Geosciences/Geologie en Mijnbouw*, no. Classic Papers.
- Rohling, E., Marino, G., and Grant, K., 2015, Mediterranean climate and oceanography, and the periodic development of anoxic events (sapropels): *Earth-Science Reviews*, v. 143, p. 62-97.
- Rohling, E. J., and Bigg, G. R., 1998, Paleosalinity and delta O-18: A critical assessment: *Journal of Geophysical Research-Oceans*, v. 103, no. C1, p. 1307-1318.
- Rohling, E. J., Hibbert, F. D., Williams, F. H., Grant, K. M., Marino, G., Foster, G. L., Hennekam, R., De Lange, G. J., Roberts, A. P., and Yu, J., 2017, Differences between the last two glacial maxima and implications for ice-sheet, $\delta^{18}\text{O}$, and sea-level reconstructions: *Quaternary Science Reviews*, v. 176, p. 1-28.
- Rohling, E. J., Sluijs, A., Dijkstra, H. A., Köhler, P., van de Wal, R. S., von der Heydt, A. S., Beerling, D. J., Berger, A., Bijl, P. K., and Crucifix, M., 2012, Making sense of palaeoclimate sensitivity: *Nature*, v. 491, no. 7426, p. 683.
- Rosenthal, Y., Lohmann, G. P., Lohmann, K. C., and Sherrell, R. M., 2000, Incorporation and preservation of Mg in *Globigerinoides sacculifer*: Implications for reconstructing the temperature and O-18/O-16 of seawater: *Paleoceanography*, v. 15, no. 1, p. 135-145.
- Rosignol-Strick, M., 1985, Mediterranean Quaternary sapropels, an immediate response of the African monsoon to variation of insolation: *Palaeogeography, palaeoclimatology, palaeoecology*, v. 49, no. 3-4, p. 237-263.
- Rosignol-Strick, M., Nesteroff, W., Olive, P., and Vergnaud-Grazzini, C., 1982, After the deluge: Mediterranean stagnation and sapropel formation: *Nature*, v. 295, no. 5845, p. 105.
- Russell, A. D., Hönisch, B., Spero, H. J., and Lea, D. W., 2004, Effects of seawater carbonate ion concentration and temperature on shell U, Mg, and Sr in cultured planktonic foraminifera: *Geochimica et Cosmochimica Acta*, v. 68, no. 21, p. 4347-4361.
- Sadekov, A., Eggins, S. M., De Deckker, P., and Kroon, D., 2008, Uncertainties in seawater thermometry deriving from intratest and intertest Mg/Ca variability in *Globigerinoides ruber*: *Paleoceanography*, v. 23, no. 1.
- Sadekov, A. Y., Bush, F., Kerr, J., Ganeshram, R., and Elderfield, H., 2014, Mg/Ca composition of benthic foraminifera *Miliolacea* as a new tool of paleoceanography: *Paleoceanography*, v. 29, no. 10, p. 990-1001.
- Sadekov, A. Y., Darling, K. F., Ishimura, T., Wade, C. M., Kimoto, K., Singh, A. D., Anand, P., Kroon, D., Jung, S., and Ganssen, G., 2016, Geochemical imprints of genotypic variants of *Globigerina bulloides* in the Arabian Sea: *Paleoceanography*, v. 31, no. 10, p. 1440-1452.
- Sadekov, A. Y., Eggins, S. M., and De Deckker, P., 2005, Characterization of Mg/Ca distributions in planktonic foraminifera species by electron microprobe mapping: *Geochemistry Geophysics Geosystems*, v. 6.
- Sanyal, A., Bijma, J., Spero, H., and Lea, D. W., 2001, Empirical relationship between pH and the boron isotopic composition of *Globigerinoides sacculifer*: Implications for the boron isotope paleo-pH proxy: *Paleoceanography*, v. 16, no. 5, p. 515-519.
- Sanyal, A., Hemming, N., Broecker, W., Lea, D. W., Spero, H. J., and Hanson, G. N., 1996, Oceanic pH control on the boron isotopic composition of foraminifera: evidence from culture experiments: *Paleoceanography*, v. 11, no. 5, p. 513-517.
- Schiebel, R., 2002, Planktic foraminiferal sedimentation and the marine calcite budget: *Global Biogeochemical Cycles*, v. 16, no. 4, p. 3-1-3-21.
- Schmidt, M. W., Vautravers, M. J., and Spero, H. J., 2006, Rapid subtropical North Atlantic salinity oscillations across Dansgaard-Oeschger cycles: *Nature*, v. 443, no. 7111, p. 561.

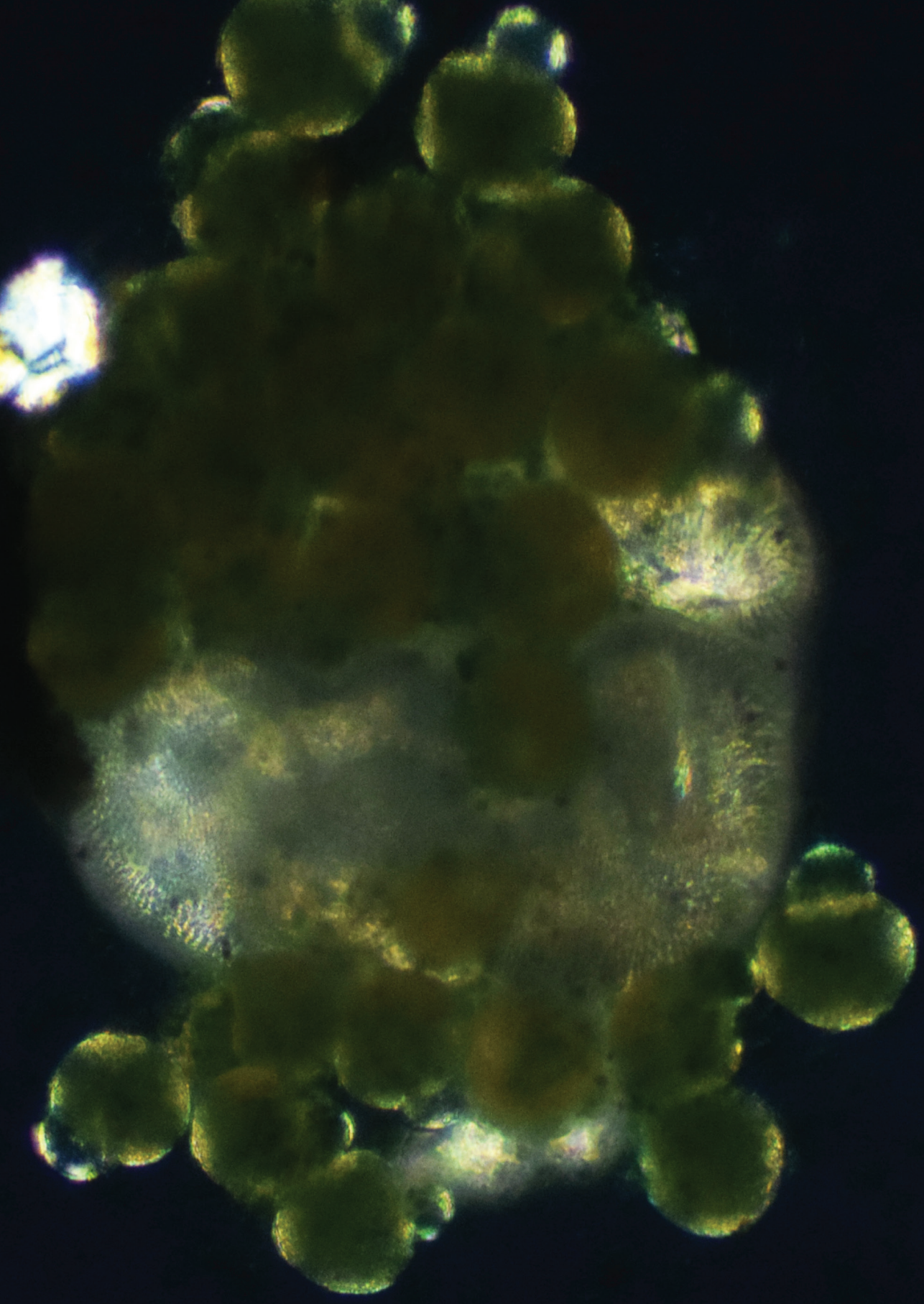
- Segev, E., and Erez, J., 2006, Effect of Mg/Ca ratio in seawater on shell composition in shallow benthic foraminifera: *Geochemistry Geophysics Geosystems*, v. 7.
- Shackleton, N., 1974, Attainment of isotopic equilibrium between ocean water and the benthonic foraminifera genus *Uvigerina*: isotopic changes in the ocean during the last glacial.
- Sinclair, D., Williams, B., and Risk, M., 2006, A biological origin for climate signals in corals—Trace element “vital effects” are ubiquitous in Scleractinian coral skeletons: *Geophysical Research Letters*, v. 33, no. 17.
- Sinclair, D. J., and Risk, M. J., 2006, A numerical model of trace-element coprecipitation in a physicochemical calcification system: Application to coral biomineralization and trace-element ‘vital effects’: *Geochimica et cosmochimica acta*, v. 70, no. 15, p. 3855-3868.
- Sluijs, A., Schouten, S., Pagani, M., Woltering, M., Brinkhuis, H., Damsté, J. S. S., Dickens, G. R., Huber, M., Reichart, G.-J., and Stein, R., 2006, Subtropical Arctic Ocean temperatures during the Palaeocene/Eocene thermal maximum: *Nature*, v. 441, no. 7093, p. 610.
- Spero, H. J., 1988, Ultrastructural examination of chamber morphogenesis and biomineralization in the planktonic foraminifer *Orbulina universa*: *Marine Biology*, v. 99, no. 1, p. 9-20.
- Spero, H. J., Eggins, S. M., Russell, A. D., Vetter, L., Kilburn, M. R., and Honisch, B., 2015, Timing and mechanism for intratest Mg/Ca variability in a living planktic foraminifer: *Earth and Planetary Science Letters*, v. 409, p. 32-42.
- Spindler, M., 1978, The development of the organic lining in *Heterostegina depressa* (Nummulitidae; Foraminifera): *The Journal of Foraminiferal Research*, v. 8, no. 3, p. 258-261.
- Sprovieri, M., Di Stefano, E., Incarbona, A., Manta, D. S., Pelosi, N., d’Alcalà, M. R., and Sprovieri, R., 2012, Centennial-to millennial-scale climate oscillations in the Central-Eastern Mediterranean Sea between 20,000 and 70,000 years ago: evidence from a high-resolution geochemical and micropaleontological record: *Quaternary Science Reviews*, v. 46, p. 126-135.
- Steffensen, J. P., Andersen, K. K., Bigler, M., Clausen, H. B., Dahl-Jensen, D., Fischer, H., Goto-Azuma, K., Hansson, M., Johnsen, S. J., and Jouzel, J., 2008, High-resolution Greenland ice core data show abrupt climate change happens in few years: *Science*, v. 321, no. 5889, p. 680-684.
- Steinhardt, J., Cléroux, C., Ullgren, J., de Nooijer, L., Durgadoo, J. V., Brummer, G.-J., and Reichart, G.-J., 2014, Anti-cyclonic eddy imprint on calcite geochemistry of several planktonic foraminiferal species in the Mozambique Channel: *Marine Micropaleontology*, v. 113, p. 20-33.
- Steinhardt, J., de Nooijer, L. L., Brummer, G. J., and Reichart, G. J., 2015, Profiling planktonic foraminiferal crust formation: *Geochemistry, Geophysics, Geosystems*, v. 16, no. 7, p. 2409-2430.
- Stoll, M. H. C., Bakker, K., Nobbe, G. H., and Haese, R. R., 2001, Continuous-flow analysis of dissolved inorganic carbon content in seawater: *Analytical Chemistry*, v. 73, no. 17, p. 4111-4116.
- Stouff, V., Debenay, J.-P., and Lesourd, M., 1999, Origin of double and multiple tests in benthic foraminifera: observations in laboratory cultures: *Marine Micropaleontology*, v. 36, no. 4, p. 189-204.
- Tang, J., Dietzel, M., Böhm, F., Köhler, S. J., and Eisenhauer, A., 2008, Sr²⁺/Ca²⁺ and ⁴⁴Ca/⁴⁰Ca fractionation during inorganic calcite formation: II. Ca isotopes: *Geochimica et Cosmochimica Acta*, v. 72, no. 15, p. 3733-3745.
- ter Kuile, B., and Erez, J., 1984, In situ growth rate experiments on the symbiont-bearing foraminifera *Amphistegina lobifera* and *Amphisorus hemprichii*: *The Journal of Foraminiferal Research*, v. 14, no. 4, p. 262-276.

- Terakado, Y., Ofuka, Y., and Tada, N., 2010, Rare earth elements, Sr, Ba, Fe, and major cation concentrations in some living foraminiferal tests collected from Iriomote Island, Japan: An exploration for trace element behavior during biogenic calcium carbonate formation: *Geochemical Journal*, v. 44, no. 4, p. 315-322.
- Tesoriero, A. J., and Pankow, J. F., 1996, Solid solution partitioning of Sr^{2+} , Ba^{2+} , and Cd^{2+} to calcite: *Geochimica et Cosmochimica Acta*, v. 60, no. 6, p. 1053-1063.
- Thomson, J., Mercione, D., De Lange, G., and Van Santvoort, P., 1999, Review of recent advances in the interpretation of eastern Mediterranean sapropel S1 from geochemical evidence: *Marine Geology*, v. 153, no. 1-4, p. 77-89.
- Thunell, R. C., and Williams, D. F., 1989, Glacial-Holocene salinity changes in the Mediterranean Sea: hydrographic and depositional effects: *Nature*, v. 338, no. 6215, p. 493.
- Toyofuku, T., Kitazato, H., Kawahata, H., Tsuchiya, M., and Nohara, M., 2000, Evaluation of Mg/Ca thermometry in foraminifera: Comparison of experimental results and measurements in nature: *Paleoceanography*, v. 15, no. 4, p. 456-464.
- Toyofuku, T., Matsuo, M. Y., de Nooijer, L. J., Nagai, Y., Kawada, S., Fujita, K., Reichart, G.-J., Nomaki, H., Tsuchiya, M., Sakaguchi, H., and Kitazato, H., 2017, Proton pumping accompanies calcification in foraminifera: *Nature Communications*, v. 8, p. 14145.
- Toyofuku, T., Suzuki, M., Suga, H., Sakai, S., Suzuki, A., Ishikawa, T., de Nooijer, L. J., Schiebel, R., Kawahata, H., and Kitazato, H., 2011, Mg/Ca and $\delta^{18}\text{O}$ in the brackish shallow-water benthic foraminifer *Ammonia 'beccarii'*: *Marine Micropaleontology*, v. 78, no. 3, p. 113-120.
- Tyszka, J., Bickmeyer, U., Raitzsch, M., Bijma, J., Kaczmarek, K., Mewes, A., Topa, P., and Janse, M., 2019, Form and function of F-actin during biomineralization revealed from live experiments on foraminifera: *Proceedings of the National Academy of Sciences*, v. 116, no. 10, p. 4111-4116.
- Urey, H. C., 1947, The thermodynamic properties of isotopic substances: *Journal of the Chemical Society (Resumed)*, p. 562-581.
- Urey, H. C., Lowenstam, H. A., Epstein, S., and McKinney, C. R., 1951, Measurement of paleotemperatures and temperatures of the upper cretaceous of England, Denmark, and the Southeastern United-States: *Geological Society of America Bulletin*, v. 62, no. 4, p. 399-&.
- van Aken, H. M., 2008, Variability of the water temperature in the western Wadden Sea on tidal to centennial time scales: *Journal of Sea Research*, v. 60, no. 4, p. 227-234.
- van der Meer, M. T., Baas, M., Rijpstra, W. I. C., Marino, G., Rohling, E. J., Damsté, J. S. S., and Schouten, S., 2007, Hydrogen isotopic compositions of long-chain alkenones record freshwater flooding of the Eastern Mediterranean at the onset of sapropel deposition: *Earth and Planetary Science Letters*, v. 262, no. 3-4, p. 594-600.
- van der Zwaan, G., Duijnste, I., Den Dulk, M., Ernst, S., Jannink, N., and Kouwenhoven, T., 1999, Benthic foraminifera: proxies or problems?: a review of paleoecological concepts: *Earth-Science Reviews*, v. 46, no. 1-4, p. 213-236.
- van Dijk, I., Barras, C., de Nooijer, L. J., Mouret, A., Geerken, E., Oron, S., and Reichart, G. J., 2018, Coupled Ca and inorganic carbon uptake suggested by magnesium and sulfur incorporation in foraminiferal calcite: *Biogeosciences Discuss.*, v. 2018, p. 1-28.
- van Dijk, I., Barras, C., Nooijer, L. J. d., Mouret, A., Geerken, E., Oron, S., and Reichart, G.-J., 2019, Coupled calcium and inorganic carbon uptake suggested by magnesium and sulfur incorporation in foraminiferal calcite: *Biogeosciences*, v. 16, no. 10, p. 2115-2130.
- van Dijk, I., de Nooijer, L. J., Boer, W., and Reichart, G. J., 2017a, Sulfur in foraminiferal calcite as a potential proxy for seawater carbonate ion concentration: *Earth and Planetary Science Letters*, v. 470, p. 64-72.

- van Dijk, I., de Nooijer, L. J., and Reichart, G.-J., 2017b, Trends in element incorporation in hyaline and porcelaneous foraminifera as a function of $p\text{CO}_2$: *Biogeosciences*, v. 14, no. 3, p. 497.
- van Dijk, I., de Nooijer, L. J., Wolthers, M., and Reichart, G.-J., 2017c, Impacts of pH and $[\text{CO}_3^{2-}]$ on the incorporation of Zn in foraminiferal calcite: *Geochimica et Cosmochimica Acta*, v. 197, p. 263-277.
- van Heuven, S., Pierrot, D., Rae, J., Lewis, E., and Wallace, D., 2011, MATLAB program developed for CO2 system calculations: Oak Ridge, Tennessee: Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, US Department of Energy. pp. ORNL/CDIAC-105b.
- van Santvoort, P., De Lange, G., Thomson, J., Cussen, H., Wilson, T., Krom, M., and Ströhle, K., 1996, Active post-depositional oxidation of the most recent sapropel (S1) in sediments of the eastern Mediterranean Sea: *Geochimica et Cosmochimica Acta*, v. 60, no. 21, p. 4007-4024.
- Vergnaud-Grazzini, C., Ryan, W. B., and Cita, M. B., 1977, Stable isotopic fractionation, climate change and episodic stagnation in the eastern Mediterranean during the late Quaternary: *Marine Micropaleontology*, v. 2, p. 353-370.
- Vigier, N., Gislason, S. R., Burton, K., Millot, R., and Mokadem, F., 2009, The relationship between riverine lithium isotope composition and silicate weathering rates in Iceland: *Earth and Planetary Science Letters*, v. 287, no. 3-4, p. 434-441.
- von der Heydt, A., S, Dijkstra, H. A., van de Wal, R. S., Caballero, R., Crucifix, M., Foster, G. L., Huber, M., Köhler, P., Rohling, E., and Valdes, P. J., 2016, Lessons on climate sensitivity from past climate changes: *Current Climate Change Reports*, v. 2, no. 4, p. 148-158.
- von Langen, P. J., Johnson, K. S., Coale, K. H., and Elrod, V. A., 1997, Oxidation kinetics of manganese (II) in seawater at nanomolar concentrations: *Geochimica et Cosmochimica Acta*, v. 61, no. 23, p. 4945-4954.
- Weber, E., and Pokroy, B., 2015, Intracrystalline inclusions within single crystalline hosts: from biomineralization to bio-inspired crystal growth: *CrystEngComm*, v. 17, no. 31, p. 5873-5883.
- Wehausen, R., and Brumsack, H.-J., 2000, Chemical cycles in Pliocene sapropel-bearing and sapropel-barren eastern Mediterranean sediments: *Palaeogeography, Palaeoclimatology, Palaeoecology*, v. 158, no. 3-4, p. 325-352.
- Weiner, S., and Dove, P. M., 2003, An overview of biomineralization processes and the problem of the vital effect: *Biomineralization*, v. 54, p. 1-29.
- Weiner, S., and Erez, J., 1984, Organic matrix of the shell of the foraminifer, *Heterostegina depressa*: *The Journal of Foraminiferal Research*, v. 14, no. 3, p. 206-212.
- Weiner, S., Levi-Kalishman, Y., Raz, S., and Addadi, L., 2003, Biologically formed amorphous calcium carbonate: *Connective Tissue Research*, v. 44, no. 1, p. 214-218.
- Weldeab, S., Lea, D. W., Schneider, R. R., and Andersen, N., 2007, 155,000 years of West African monsoon and ocean thermal evolution: *science*, v. 316, no. 5829, p. 1303-1307.
- Weldeab, S., Menke, V., and Schmiedl, G., 2014, The pace of East African monsoon evolution during the Holocene: *Geophysical Research Letters*, v. 41, no. 5, p. 1724-1732.
- Weldeab, S., Schneider, R. R., and Kölling, M., 2006, Comparison of foraminiferal cleaning procedures for Mg/Ca paleothermometry on core material deposited under varying terrigenous-input and bottom water conditions: *Geochemistry, Geophysics, Geosystems*, v. 7, no. 4.
- Weltje, G. J., Bloemsma, M., Tjallingii, R., Heslop, D., Röhl, U., and Croudace, I. W., 2015, Prediction of geochemical composition from XRF core scanner data: a new multivariate approach including automatic selection of calibration samples and quantification of uncertainties, *Micro-XRF Studies of Sediment Cores*, Springer, p. 507-534.
- White, A. F., 1978, Sodium coprecipitation in calcite and dolomite: *Chemical Geology*, v. 23, no. 1, p. 65-72.

References

- Wit, J. C., de Nooijer, L. J., Barras, C., Jorissen, F. J., and Reichart, G. J., 2012, A reappraisal of the vital effect in cultured benthic foraminifer *Bulimina marginata* on Mg/Ca values: assessing temperature uncertainty relationships: *Biogeosciences*, v. 9, no. 9, p. 3693-3704.
- Wit, J. C., de Nooijer, L. J., Wolthers, M., and Reichart, G. J., 2013, A novel salinity proxy based on Na incorporation into foraminiferal calcite: *Biogeosciences*, v. 10, no. 10, p. 6375-6387.
- Wolf-Gladrow, D. A., Bijma, J., and Zeebe, R. E., 1999, Model simulation of the carbonate chemistry in the microenvironment of symbiont bearing foraminifera: *Marine Chemistry*, v. 64, no. 3, p. 181-198.
- Yoshimura, T., Tamenori, Y., Suzuki, A., Kawahata, H., Iwasaki, N., Hasegawa, H., Nguyen, L. T., Kuroyanagi, A., Yamazaki, T., and Kuroda, J., 2017, Altrivalent substitution of sodium for calcium in biogenic calcite and aragonite: *Geochimica et Cosmochimica Acta*, v. 202, p. 21-38.
- Yu, J., Elderfield, H., Greaves, M., and Day, J., 2007, Preferential dissolution of benthic foraminiferal calcite during laboratory reductive cleaning: *Geochemistry, Geophysics, Geosystems*, v. 8, no. 6.
- Zachos, J. C., Schouten, S., Bohaty, S., Quattlebaum, T., Sluijs, A., Brinkhuis, H., Gibbs, S., and Bralower, T., 2006, Extreme warming of mid-latitude coastal ocean during the Paleocene-Eocene Thermal Maximum: Inferences from TEX86 and isotope data: *Geology*, v. 34, no. 9, p. 737-740.
- Zeebe, R. E., and Sanyal, A., 2002, Comparison of two potential strategies of planktonic foraminifera for house building: Mg^{2+} or H^+ removal?: *Geochimica et Cosmochimica Acta*, v. 66, no. 7, p. 1159-1169.
- Zeebe, R. E., Sanyal, A., Ortiz, J. D., and Wolf-Gladrow, D. A., 2001, A theoretical study of the kinetics of the boric acid–borate equilibrium in seawater: *Marine Chemistry*, v. 73, no. 2, p. 113-124.
- Zeebe, R. E., and Westbroek, P., 2003, A simple model for the $CaCO_3$ saturation state of the ocean: The “Strangelove,” the “Neritan,” and the “Cretan” Ocean: *Geochemistry, Geophysics, Geosystems*, v. 4, no. 12.
- Zeebe, R. E., Wolf-Gladrow, D. A., Bijma, J., and Hönisch, B., 2003, Vital effects in foraminifera do not compromise the use of $\delta^{11}B$ as a paleo-pH indicator: Evidence from modeling: *Paleoceanography*, v. 18, no. 2.
- Zhang, Y., and Dawe, R. A., 2000, Influence of Mg^{2+} on the kinetics of calcite precipitation and calcite crystal morphology: *Chemical Geology*, v. 163, no. 1-4, p. 129-138.
- Ziegler, M., Tuenter, E., and Lourens, L. J., 2010, The precession phase of the boreal summer monsoon as viewed from the eastern Mediterranean (ODP Site 968): *Quaternary Science Reviews*, v. 29, no. 11-12, p. 1481-1490.
- Zwiep, K. L., Hennekam, R., Donders, T. H., Van Helmond, N. A., De Lange, G. J., and Sangiorgi, F., 2018, Marine productivity, water column processes and seafloor anoxia in relation to Nile discharge during sapropels S1 and S3: *Quaternary Science Reviews*, v. 200, p. 178-190.



Addendum

Summary
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"The far-off land may have bays, forelands, angles in and out to any number and extend;
yet at a distance you see none of these, nothing but a grey unbroken line upon the water."

- Flatland: a romance of many dimensions, Edwin A. Abbott

Summary

Accurate reconstructions of climate system parameters throughout geological history help constraining climate sensitivity to anthropogenic CO₂ and thereby allow predicting climate change in the (near) future. The oceans play a fundamental role in the climate system and improved estimates of past seawater salinity and temperature are necessary to reconstruct variability in overturning circulation and the hydrological cycle. Foraminifera are unicellular marine organisms that are globally ubiquitous and abundant throughout the geological record. The calcite chemistry of fossil foraminiferal shells reflect seawater conditions during the time of formation and is therefore an excellent tool (i.e. a proxy) to reconstruct ocean variables. Studies linking incorporation of trace elements in foraminiferal calcite to seawater variables have expanded and improved the paleoceanographic proxy toolbox, with e.g. Mg/Ca as a proxy for temperature, Na/Ca for salinity and Sr/Ca for seawater carbonate chemistry. However, ever since the first application of proxies based on foraminiferal shell chemistry, it has been evident that the organism itself exerts a strong additional control on element incorporation. This fundamentally hampers a one-to-one comparison of relationships obtained for inorganically precipitated calcite and foraminiferal calcite and hence the thermodynamic underpinning of proxy relationships. Acknowledging this gap in our understanding, the overarching aim of this thesis is to better understand foraminiferal trace element incorporation and variability therein, and to constrain to which extend element incorporation is controlled by the organism and by the environment. To disentangle these two controls, variability in element to calcium ratios (El/Ca) is studied at different scales: within the shell wall, between specimens and between species. Patterns in El/Ca variability across scales will be related to changes in environmental conditions to improve their value in their application to the geological past.

Culture- and labeling studies with living foraminifera are central in this thesis; as such experiments allow modification of environmental parameters under controlled conditions, thereby isolating the effect of individual parameters, which are coupled in the natural environment. A recently proposed proxy for salinity, Na/Ca, was tested by growing foraminiferal individuals of the benthic low-Mg/Ca species *Ammonia tepida* and intermediate-Mg/Ca species *Amphistegina lessonii* at salinities between 20 and 40. Between salinities 20 and 40, Na/Ca shows a 0.064 (for *A. tepida*) and 0.077 mmol/mol (for *A. lessonii*) increase per salinity unit, although inter-specimen variability is larger than the salinity effect. We show that elements are correlated between specimens and that incorporated Na and Mg are also correlated within chamber walls of both species, whereby electron microprobe maps show that Na and Mg occur in bands of

elevated concentrations. We suggest that inter-species, inter-specimen and intra-shell variability in El/Ca, are all caused by (variability in) the organismal controls during biomineralization. Understanding this organismal control is necessary to improve reliability of proxies based on these elements.

Therefore, element banding within chamber walls is studied in detail using Nanoscale-Secondary Ion Mass Spectrometry (NanoSIMS). Specimens of the low-Mg species *Ammonia tepida* and of the intermediate-Mg species *Amphistegina lessonii* were grown at different temperatures and salinities, to assess how these parameters affect the high- and low element-bands (peaks and troughs, respectively) within the shell wall. We show that both El/Ca peaks and troughs are raised in specimens grown at a higher temperature or salinity, implying that these environmental parameters have a consistent effect on average El/Ca values despite the heterogeneous element distribution within the shell wall. The correlated El/Ca peak and trough heights observed here between specimens further support a biomineralization mechanism simultaneously controlling the incorporation of different elements during chamber formation.

A poorly quantified, but potentially key driver within foraminiferal biomineralization, is the rate of calcite precipitation. To obtain this rate we use Sr to label the shell wall during the precipitation of a single chamber and subsequent visualization of the incorporated Sr-label with Nano-SIMS. Results show that precipitation rates are surprisingly uniform between specimens at $\sim 22 \pm 2$ nmol/cm²/min, comparable to the fastest rates obtained by inorganic calcite precipitation experiments. This may explain foraminiferal Sr incorporation as precipitation from a Mg-free seawater like solution, since Sr partitioning in foraminifera matches that in inorganic precipitation performed with a similar rate as that found here. Moreover, these results aid understanding of the role of precipitation rate (i.e. kinetics) on partitioning of other trace element. In addition, we show that the precipitation rate is significantly lower in the first layer of calcite, compared to the rest of the shell wall. This may be explained by the presence of inhibitors like magnesium- and sulphate ions at the onset of calcification, of which concentrations are subsequently diluted by sustained inward transport of Ca²⁺ during calcification.

To address different factors impacting element incorporation and test various novel El/Ca-based proxies down-core, a piston-core was retrieved from the Eastern Mediterranean Sea, covering the last 350,000 years. During this time interval, pronounced changes in sea surface salinity and temperature, driven by changes in Northern Hemisphere insolation leading to monsoon variability and hence river runoff, led to intermitted deposition of dark, organic-rich sediments. These so-called sapropels represent episodes of enhanced

productivity and bottom water deoxygenation. We show that increased foraminiferal Ba/Ca and Mn/Ca reflect seawater freshening in conjunction with sapropels, likely due to increased Nile-river runoff. We conclude that Na/Ca is not a suitable recorder of salinity down-core in the setting studied here, yet $\Delta\text{Mg}/\text{Ca}$ appears a promising candidate as a salinity proxy when large changes in salinity are involved.

Finally, we synthesize observations of El/Ca variability at different scales and hypotheses on the biomineralization mechanisms involved, presented throughout this thesis, by combining and extending existing biomineralization models for element incorporation. A numerical model for element incorporation as a function of shell wall thickness, based on precipitation of calcite from a confined space of a specified volume and involving inward Ca^{2+} -transport and inorganic partitioning coefficients, is presented and compared to observations. Our model is able to explain average element incorporation and banding therein for two species studied in this thesis (*A. tepida* and *A. lessonii*). Using the model's outcome, differences between these species can be explained by variability in the volume of the calcifying fluid and rate of inward Ca^{2+} -pumping.

Nederlandse samenvatting

Het klimaat op aarde is momenteel hard aan het veranderen. Om te kunnen voorspellen hoe warm het wordt bij een gegeven toename in kooldioxide (CO_2), kan het verleden als een testcase gebruikt worden. Nauwkeurige reconstructies van vroegere klimaatveranderingen kunnen vertellen hoe sterk het klimaat reageert op bijvoorbeeld natuurlijke variaties in CO_2 . In zulke veranderingen spelen de oceanen een cruciale rol. Het zoutgehalte en de temperatuur van het zeewater door de tijd heen zijn bijvoorbeeld de aandrijvers van schommelingen in de thermohaline circulatie en de hydrologische cyclus.

De chemische samenstelling van het van fossiele kalkschelpjes van foraminifera wordt bepaald door de condities tijdens de bouw van deze schelpjes. Daardoor dienen ze als een methode (ofwel: proxy) om oceaan- en klimaatvariabelen, zoals temperatuur en zoutgehalte, te reconstrueren. Zulke reconstructies bestaan bij de gratie van veldstudies en experimenten waarin de inbouw van (spoor-) elementen en fractionatie van isotopen in het kalk van foraminiferen als functie van zeewater-variabelen worden gekwantificeerd. Zo blijkt Mg/Ca bijvoorbeeld een proxy voor zeewater temperatuur, Na/Ca voor zoutgehalte en Sr/Ca voor de anorganische koolstof chemie van het zeewater te zijn. Met de toename van onze kennis over proxies gebaseerd op de chemie van deze schelpjes, is het duidelijk geworden dat naast omgevingsfactoren zoals temperatuur, het organisme zelf ook een sterke invloed heeft op de inbouw van elementen. Het effect van deze zogenaamde biomineralizatie staat een directe vergelijking in de weg tussen omgevingsfactoren en element inbouw zoals die bekend zijn op basis van chemische en fysische wetmatigheden die gelden tijdens calcium carbonaat precipitatie. Het doel van dit proefschrift is om beter te begrijpen hoe foraminiferen de inbouw van elementen beïnvloeden en of de biologische activiteit de natuurlijke variatie in element-samenstelling kan verklaren. Hiervoor heb ik de variatie in element concentraties (uitgedrukt als ratio ten opzichte van calcium; de El/Ca) van het calcië van schelpjes op verschillende schalen bestudeerd: tussen verschillende soorten, tussen individuen, en binnenin de kamer wand van de schelp.

Cultuur- en labelling studies met levende foraminiferen vormen de basis van mijn proefschrift. Het grote voordeel van deze aanpak is dat de omgeving-condities onafhankelijk van elkaar gemanipuleerd kunnen worden, terwijl in de natuur omgevingsfactoren juist gezamenlijk variëren. Een recent ontwikkelde proxy voor het zoutgehalte van de zee is de Na/Ca ratio, en werd hier getest door foraminifera van de soort *Ammonia tepida* (voorkomend in gematigd klimaat en een zogenaamd laag-Mg

calciet producerend) en *Amphistegina lessonii* (tropisch klimaat, gemiddelde Mg-calciet) te laten groeien in zeewater met zoutgehaltes variërend van 20 tot 40. We laten zien dat over dit bereik, Na/Ca toeneemt met 0.064 (*A. tepida*) en 0.077 (*A. lessonii*) millimol per mol per saliniteits-unit. Het bleek echter ook zo te zijn dat de variabiliteit in Na/Ca tussen individuen binnen één conditie groter is dan het (gemiddelde) verschil in Na/Ca tussen de condities. Ik beschrijf ook dat de concentraties van verschillende (spoor)-elementen (Sr/Ca, Mg/Ca en Na/Ca) gecorreleerd zijn tussen de individuen en dat de inbouw van Na en Mg ook gecorreleerd is binnen de wand van een kamer. Dat laatste is vooral duidelijk uit electron microprobe maps die laten zien dat Na en Mg concentraties verhoogd zijn in zones rondom de organische laag waarop het eerste calciet geprecipiteerd wordt. Deze resultaten suggereren dat variatie in de element inbouw tussen soorten, individuen en binnen de kamer muur van de schelp veroorzaakt wordt door (variabiliteit in) de controle van het organisme tijdens de biomineralizatie.

De verdeling van elementen binnen de wand van de schaaltes van foraminiferen, die vooral te zien is als zones van afwisselend hogere en lagere concentraties, is in detail bestudeerd met Nanoscale-Secondary Ion Mass Spectrometry (NanoSIMS). Individuele schelpjes van *Ammonia tepida* en *Amphistegina lessonii* zijn daarvoor gegroeid bij verschillende temperaturen en zoutgehaltes, om te onderzoeken wat het effect van deze condities is op de element verdeling binnen dwarsdoorsneden van de schelpwand. Ik beschrijf verder dat de concentraties van zowel de zones met veel, als die met weinig El/Ca toenemen met temperatuur of zoutgehalte. Dit suggereert dat deze parameters dus een consistent effect hebben op de gemiddelde El/Ca waarden van foraminifera schelpjes, ondanks de heterogene element verdeling binnen de wand van het schelpje. Daarnaast blijkt dat de El/Ca van zowel pieken als dalen zijn gecorreleerd tussen de individuen, wat suggereert dat er een onderliggend biomineralizatie mechanisme verantwoordelijk is voor de inbouw van deze elementen.

Een relatief onbekende, maar potentieel belangrijke factor tijdens biomineralizatie, is de snelheid waarmee het calciet precipiteert tijdens de kamerbouw. Om deze snelheid te meten hebben we het element Sr gebruikt om de schelpwand te markeren tijdens de vorming daarvan. Vervolgens zijn de ingebouwde labels in de dwarsdoorsnede van de kamer gevisualiseerd met NanoSIMS. Deze resultaten laten zien dat de snelheid waarmee het calciet precipiteert onverwacht hoog is en constant is tussen individuen, met $\sim 22 \pm 2$ nmol/cm²/min. Deze waarde is vergelijkbaar met de hoogste snelheden gemeten in experimenten waarbij anorganische (niet-biologisch) calciet werd gevormd. Deze hoge precipitatie snelheid kan de Sr concentratie (en mogelijk andere elementen) in foraminifera verklaren op basis van chemisch-fysische principes. Verder laten we

zien dat de precipitatie snelheid significant lager is in de eerste fase van de kamer bouw, wat verklaart zou kunnen worden door de aanwezigheid van ionen die de groei van calciet remmen. Dit zou bijvoorbeeld kunnen komen door relatief hoge concentraties magnesium en sulfaat aan het begin van de kamerbouw, waarvan de concentratie geleidelijk afneemt tijdens latere stadia van kamerbouw wanneer er meer Ca²⁺ naar de calcificatie-ruimte wordt gepompt.

Om verschillende El/Ca proxies te testen en meerdere klimaat-factoren te onderzoeken die element inbouw in de schelpen van foraminifera beïnvloeden, hebben we een kern genomen in de Oostelijke Middellandse Zee, die sedimenten bevat met een ouderdom tot 350,000 jaar terug in de tijd. Gedurende dit tijdsinterval waren er sterke schommelingen in de temperatuur van het zeewater en het zoutgehalte van het oppervlakte water. Deze schommelingen werden aangedreven door veranderingen in de zonnestraling (insolatie), wat zorgde voor variabiliteit in de moessons en afvoer door de Nijl. Tijdens perioden van toegenomen rivier afvoer werden donkere, organisch-rijke sedimenten afgezet, zogenoemde 'sapropels', door de toegenomen productiviteit en zuurstofloosheid van het zeebodem water. We laten zien dat Ba/Ca en Mn/Ca in foraminifera schelpen verhoogd is tijdens deze perioden van hevige moessons en toegenomen rivier afvoer. We concluderen dat Na/Ca in deze kern geen betrouwbare proxy is voor het zoutgehalte van het zeewater, Δ Mg/Ca lijkt echter wel een veelbelovende kandidaat, wanneer er grote fluctuaties zijn in het zoutgehalte over de tijd.

Tenslotte brengen we de observaties van El/Ca variabiliteit op verschillende niveaus en hypothesen over de biomineralizatie mechanismen, die hiervoor verantwoordelijk zijn, samen in het laatste hoofdstuk van dit proefschrift. Dit doe ik door bestaande modellen voor element inbouw in foraminifera schelpjes te combineren, uit te breiden en te vergelijken met observaties. Ik presenteer een numeriek biomineralizatie model van element inbouw als functie van wanddikte, gebaseerd op het idee dat het calciet geprecipiteerd wordt binnen een afgesloten ruimte met een gespecificeerd volume, waarbij Ca²⁺ naar binnen wordt getransporteerd. Resultaten laten zien dat het verschil in El/Ca tussen individuen en tussen de twee foraminifera soorten *A. tepida* en *A. lessonii* verklaard kan worden door verschillen in het volume van de calcificatie ruimte. Variabiliteit in El/Ca binnen de kamerwand kan verklaard door het insluiten van zeewater, dat deze elementen bevat, aan het begin van de kamerbouw en het daarna verdunnen van de El/Ca concentratie door het naar binnen transporteren van Ca²⁺.

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About the author

Esmee Geerken was born on the 27th of July 1985 in Eindhoven, the Netherlands. While growing up, ocean sciences did not occupy her mind a great deal as the sea was far away and the terrestrial world was mind-blowing enough. She did however like shells and at the age of 8 she wrote to *Willem Wever* to ask him “how do shells make babies”. He never answered. Nevertheless, Esmee started a somewhat non-linear journey to figure out the private affairs of shells. In 2004, she started a bachelor’s degree in audiovisual arts at the *Gerrit Rietveld Academy*, graduating with a project involving the curvature of the Earth. She then enrolled in the interdisciplinary bachelor ‘Beta-Gamma’, with a major in Earth Science, at the *University of Amsterdam* in 2008. After digging many man-deep holes to study the soil and modelling how sea and wind currents affected the drifting paths of seagulls, she longed for sediments beyond the reach of the ordinary shovel, vaster ocean currents and especially deep time. This was offered in the masters ‘Applied Environmental Geosciences’ at the *Vrije Universiteit van Amsterdam* where she graduated in 2014 (cum laude). During her masters, shells finally reentered her life with a research project about the buoyancy mechanisms of foraminiferal shells. The promise of a trans-Atlantic cruise lured her into an internship at the NIOZ, resulting in a master thesis about Deep-sea sediments of the Gambia Abyssal Plain. The prospect of shells reproducing before her own eyes (and finally getting an answer to the question left unanswered by *Willem Wever*), led her to accept a PhD position at the NIOZ, supervised by Pr. Dr. Gert-Jan Reichart and Dr. Lennart de Nooijer. During her PhD project, she participated in the Urbino summer school for paleoclimatology, the NESSC Mediterranean research cruise, was teaching assistant for the master course ‘Paleoceanography’, and conducted experiments at the Caribbean Netherlands Science Institute on St. Eustatius and the Japanese Agency for Marine and Science and Technology in Japan.



Bibliography

Geerken, E., Nooijer, L. J. D., Dijk, I. V., & Reichart, G. J. (2018). Impact of salinity on element incorporation in two benthic foraminiferal species with contrasting magnesium contents. *Biogeosciences*, 15(7), 2205-2218.

Geerken, E., de Nooijer, L. J., Roepert, A., Polerecky, L., King, H. E., & Reichart, G. J. (2019). Element banding and organic linings within chamber walls of two benthic foraminifera. *Scientific reports*, 9(1), 3598.

Dirksen, J. P., Hennekam, R., **Geerken, E.**, & Reichart, G. J. (2019). A Novel Approach Using Time-Depth Distortions to Assess Multicentennial Variability in Deep-Sea Oxygen Deficiency in the Eastern Mediterranean Sea During Sapropel S5. *Paleoceanography and Paleoclimatology*.

Dijk, I. V., Barras, C., Nooijer, L. J. D., Mouret, A., **Geerken, E.**, Oron, S., & Reichart, G. J. (2019). Coupled calcium and inorganic carbon uptake suggested by magnesium and sulfur incorporation in foraminiferal calcite. *Biogeosciences*, 16(10), 2115-2130.

In review, submitted or in preparation

Geerken, E., de Nooijer, L. J., Toyofuku, T., Roepert, A., Middelburg, J.J., Nagai, Y., Polerecky, L. & Reichart, G. J. High precipitation rates characterize biomineralization in foraminifera. (*In review at PNAS*).

Roepert, A., Polerecky, L., **Geerken, E.**, Gert-Jan Reichart, Middelburg, J.J., Distribution of chlorine and fluorine in benthic foraminifera. (*Submitted to Biogeosciences*).

Geerken, E., Nooijer, L. J., Hennekam, R., Kruithof, L., & Reichart, G. J. Nile river discharge and temperature in the Eastern Mediterranean reflected in foraminiferal stable isotope and trace metal incorporation. (*In prep*).

"Ik droomde, dat ik langzaam leefde...
 langzamer dan de oudste steen.
Het was verschrikkelijk: om mij heen
 schoot alles op, schokte of beefde,
wat stil lijkt. 'k Zag de drang waarmee
 de bomen zich uit de aarde wrongen
 terwijl ze hees en hortend zongen;
 terwijl de jaargetijden vlogen
 verkleurende als regenbogen...
 Ik zag de tremor van de zee,
zijn zwellen en weer haastig slinken,
 zoals een grote keel kan drinken.
En dag en nacht van korten duur
vlammen en doven: flakrend vuur.
- De wanhoop en welsprekendheid
 in de gebaren van de dingen,
 hun ademloze, wreede strijd...
Hoe kón ik dat niet eerder weten,
 niet beter zien in vroeger tijd?
Hoe moet ik het weer ooit vergeten?"

- 'Tijd', in Woestijnen en parken, M. Vasalis